5-31-2019

A Behavioral and Physiological Investigation of the Effects of Nicotine on Human Reward Responding and Fear Conditioning

Alexandra N. Palmisano
University of Connecticut - Storrs, alexandra.palmisano@uconn.edu

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Although considerable progress has been made, we do not fully understand the neuropsychological properties of nicotine that drive nicotine dependence. The present body of work provides an original investigation of the effects of nicotine on behavioral and physiological responding for rewarding and aversive stimuli in humans, which arises from considerable nonhuman investigations of nicotine's complex interactions in conditioned learning. In order to accomplish this goal, three distinct studies were conducted. Given evidence that nicotine enhances operant responding for nonpharmacological reinforcers, Study 1 assessed the ability of nicotine to enhance food reward sensitivity using a virtual reality conditioned place preference paradigm. Acute administration of nicotine prior to conditioning resulted in partial reward enhancement in that nicotine-treated participants demonstrated place preferences by spending significantly more time in the previously rewarded context, while the placebo group did not. However, we did not observe significant differences between treatment groups. Study 2 explored differences in reward responding between nicotine users and non-users. Research suggests that nicotine users assign enhanced motivational salience to nicotine rewards; however, discrepancies exist as to whether this enhanced salience modulates the reward value of non-drug cues. Using physiological measures and explicit pleasurability ratings of affectively rewarding stimuli and drug-related cues, we found that nicotine users attributed enhanced incentive salience to nicotine rewards relative to non-users. However, we found little evidence of hyporeactivity to non-nicotine rewards among nicotine users. We also examined the effects of acute nicotine administration on these measures of reward processing but found no effect of nicotine in our

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nondependent sample. Finally, to extend nonhuman findings that nicotine particularly facilitates hippocampal-dependent fear learning, Study 3 measured the effects of acute nicotine on conditioned fear using a novel virtual reality fear conditioning paradigm. We observed nicotine-enhanced contextual fear learning but found no enhancement of trace fear. Hypotheses generated by this data provide insight into the mechanisms that underlie nicotine dependence and anxiety disorder comorbidity, as well as risks for the development and maintenance of nicotine use, and risks for relapse following cessation. Ongoing research may aid in the development of behavioral and pharmacological interventions and treatments for nicotine dependence.

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Alexandra N. Palmisano

B.S. St. Lawrence University, 2011
M.S. University of Connecticut, 2016

A Dissertation
Submitted in Partial Fulfillment of the
Requirements for the Degree of
Doctor of Philosophy
at the
University of Connecticut
2019

APPROVAL PAGE

Doctor of Philosophy Dissertation

A Behavioral and Physiological Investigation of the Effects of Nicotine on Human Reward Responding and Fear Conditioning

Presented by

Alexandra N. Palmisano, B.S., M.S.

Major Advisor _____

Robert S. Astur, PhD

Associate Advisor _____

Thomas Gould, PhD

Associate Advisor _____

John Salamone PhD

Associate Advisor _____

Etan Markus, PhD

Associate Advisor _____

Ian Stevenson, PhD

ACKNOWLEDGEMENTS

This dissertation and my general well-being can be accredited to numerous wonderful people. First, my sincerest thanks to my advisor, Dr. Robert Astur, for his guidance and support throughout this process. It was an honor to be your first graduate student and to aid in the establishment of such an inspiring and invigorating laboratory. Thank you for trusting me, advocating for me, and providing me the freedom to pursue my personal research interests and develop my own sense of who I want to be as a researcher. Your lessons on navigating the sometimes-challenging and always-demanding terrain of academia, and your reminders to "just breath," will remain with me throughout my career.

I would also like to thank my committee members, Drs. Thomas Gould, John Salamone, Etan Markus, and Ian Stevenson for their expertise and unique perspectives that have helped me grow as a young researcher. Each of you have generously extended your time and knowledge to me throughout my graduate experience, and I deeply respect your advice and guidance.

Other mentors I would like to thank for advancing my knowledge and education include my 7th grade science teacher, Mr. Earl Horn, who was the first to instill in me a passion for the sciences, and my undergraduate mentors, Drs. Ana Estevez and Bill DeCoteau, who introduced me to neuroscience research and planted the initial inspiration to pursue a doctorate.

I would like to thank my fellow graduate student, Soniya Assudani-Patel, whose camaraderie (and occasional commiseration) throughout this adventure I deeply appreciate. Our teamwork in orchestrating day-to-day lab responsibilities felt effortless and I am grateful to have shared my time with you. Additionally, the completion of these dissertation experiments would not have been possible without the dedication and technical skills of the Astur lab undergraduates past and present: Callie Love, Kyrstyn Jenkins, Rob Livoti, Elizabeth Bulkley, Sophia Pellegrino, Michelle Padua, Allison Arnista, Mallory Mourmouras, Mirella Fernandez, Faith Okifo, Aleks Purins, Kristy Miller, Emma Lecky, and Gina Ciambriello. Thank you immensely to the best 'NicoTeam' I could have asked for.

Of course, none of this work would have been possible without my exceptional support system outside of academia. Thank you, Mom, for always believing in me, for our daily phone calls, and for your words of encouragement on my hardest days. Thank you, Dad, for teaching me how to articulate my ideas in writing and for instilling in me a sense of indomitable perseverance. Thank you, Nick, for the unspoken reminder that life's major challenges are conquerable. Thank you to my friends, for allowing me to be absent while selfishly pursuing this life goal and for loyally remaining by my side. And thank you, Ross, for making me immensely happy. Words cannot express the magnitude of my appreciation for your patience throughout this process and your unconditional support. This would not have happened with you, and I cannot wait to see what the future brings us.

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I. GENERAL INTRODUCTION

Abundant evidence indicates that nicotine is the principal addictive component of tobacco smoke. Tobacco use is the leading cause of preventable disease in the United States, resulting in approximately 480,000 premature deaths per year (U.S. Department of Health and Human Services, 2014). Health problems arising from tobacco use include heart disease, lung disease, and cancer, as well as an increased susceptibility to a variety of infectious diseases (U.S. Department of Health and Human Services, 2014). Despite the risks associated with tobacco use, 15.5% of United States adults currently smoke tobacco cigarettes (Centers for Disease Control and Prevention, 2018). Moreover, tobacco use is markedly higher among the most vulnerable members of society where it has been reported that 25% of U.S. adults with mental health disorders account for 40% of the tobacco cigarettes smoked in the U.S. (Substance Abuse and Mental Health Services Administration, 2013).

While the prevalence of tobacco cigarette smoking is trending downward (Centers for Disease Control and Prevention, 2017), electronic cigarette use has increased in popularity across all age groups since being introduced to the U.S. market in 2006-2007, and e-cigarettes are now the most commonly used nicotine product among youth (U.S. Department of Health and Human Services, 2016). Among middle and high school students, e-cigarette use has more than tripled since 2011 with approximately 16% of high school students reporting past-30-day use (U.S. Department of Health and Human Services, 2016). Despite being marketed as a safer alternative to tobacco cigarettes, a 2016 Report of the Surgeon General concluded that the use of any products containing nicotine poses dangers, particularly to young people, pregnant women, and fetuses (U.S. Department of Health and Human Services, 2016). Adverse consequences to fetuses include sudden infant death syndrome, malformed corpus callosum, deficits in auditory processing, and obesity. In adolescents, consequences of use include learning and attention

deficits, impaired impulse control, mood disorders, and increased vulnerability for use of other addictive substances (U.S. Department of Health and Human Services, 2016). Given their relatively recent introduction to the market, longitudinal data are needed to further characterize the health consequences of e-cigarette use and dependence, but they are currently identified as a public health concern. In addition to the detrimental impact of nicotine use on the user's health, the economic costs are also high. Between the years 2009 and 2012, the annual costs in the U.S. attributable to health-related expenses and lost worker productivity resulting from smoking were estimated to be between $289 and $332.5 billion (Maciosek et al., 2015).

Experts report that 68.8% of U.S. tobacco users want to quit (Centers for Disease Control and Prevention, 2018), but approximately 80% of smokers who attempt to quit on their own relapse within the first month of abstinence, and roughly only 3% remain abstinent at six months (Centers for Disease Control and Prevention, 2009). These statistics illustrate the severity of nicotine dependence and the chronic nature of the disorder. Thus, an understanding of how nicotine produces dependence and influences usage provides a necessary foundation for optimal nicotine use prevention and treatment therapies.

1.1. The effects of nicotine on reinforcement

Difficulty controlling nicotine use behaviors is believed to result from nicotine's acute pharmacological effects, which have been shown to relieve negative affective states, including stress, anxiety, anhedonia, and cognitive dysfunction (Benowitz, 1996, 2009; Cook et al., 2010; Patterson et al., 2010; Hall et al., 2015), and produce subjective sensations that can be described as euphoric (Henningfield et al., 1985). However, the hedonic effects of nicotine are relatively modest compared to other stimulant drugs (Balfour, 2004). For example, operant response rates are significantly lower for nicotine than for cocaine under identical conditions (Corrigall et al.,

1994; Risner & Goldberg, 1983), and rats prefer a cocaine-paired lever over a nicotine-paired lever in choice tests (Manzardo et al., 2002). Further investigations of nicotine's primary reinforcing properties suggest that rates of nicotine self-administration are low and specific to certain experimental conditions (Caggiula et al., 2002; Donny et al., 2003; Chaudhri et al., 2005, 2006). These data suggest that the intrinsically rewarding primary effects of nicotine may not be the only explanation for nicotine dependence.

An additional explanation for nicotine dependence is that nicotine enhances the rewarding properties of nicotine-associated stimuli. These stimuli can include things like the sight, smell, and taste of a cigarette, or the context in which nicotine's actions take place. The repeated association of nicotine use with these otherwise neutral stimuli constitutes a form of classical conditioning during which nicotine-paired stimuli are assigned incentive salience and start to function as drug cues. In this sense, incentive salience defines the biasing of cognitive processing toward reward cues triggering excessive motivational "wanting" for rewards (Robinson & Berridge, 1993, 2000). These cues, acting as conditioned reinforcers, thus promote nicotine-seeking behavior and use (Di Chiara, 2000). For example, pairing a neutral, non-drug stimulus with response-dependent nicotine delivery robustly facilitates nicotine self-administration, where operant response rates are low in the absence of the contingent stimulus (Caggiula et al. 2002; Donny et al. 2003; Chaudhri et al. 2005). Demonstrating this phenomenon in humans, several studies show that smokers exposed to smoking cues, like a lit cigarette resting in an ashtray, report increased cravings or "a desire to smoke" (Wertz & Sayette, 2001; Sayette & Tiffany, 2013). Moreover, studies have shown that when delivered without cigarette smoke, the effects of nicotine are less rewarding than after smoking (Henningfield & Goldberg, 1983; Perkins et al., 1992; Pomerleau et al., 1992), and both nicotine and cues are necessary to reduce

craving (Rose et al., 2000).

While the primary reinforcing effects of nicotine and the subsequent classically conditioned properties of nicotine-associated stimuli are important in maintaining drug use, other studies suggest that nicotine can also potentiate the reinforcing properties of stimuli that are not even explicitly paired with nicotine intake (Caggiula et al., 2008). For example, in a study by Donny et al. (2003), rats that self-administered nicotine explicitly paired with a visual stimulus (contingent condition), also controlled nicotine delivery to a separate group of animals that responded for the visual stimulus alone (non-contingent condition). Equally elevated response rates were found for the contingent and non-contingent conditions compared to responding for a saline-paired visual stimulus, suggesting that nicotine can also potentiate the reinforcing properties of non-drug cues through non-associative mechanisms. Nicotine administration that is neither temporally nor causally associated with behavior has also been shown to increase operant responding for food (Popke et al., 2000), alcohol (Clark et al., 2001), and cocaine (Bechtholt & Mark, 2002). These data are important because they suggest that nicotine dependence may result from the synergistic actions of nicotine's direct and indirect effects, such that cues both explicitly and inexplicitly paired with nicotine are imbued with enhanced motivational value, which amplifies the consummation of rewards. Moreover, this hypersensitivity to reward may persist even after nicotine intake has ended (Attwood et al., 2009). Given the frequent comorbidity between nicotine use and the use of other substances of abuse like alcohol or cocaine, these data have potential clinical relevance suggesting that nicotine may enhance the incentive salience of cocaine or alcohol conditioned cues (Chaudhri et al., 2006). This theory is supported by studies which demonstrate that nicotine enhances cue-elicited cocaine craving (Reid et al., 1998), and that cravings can be attenuated by nicotinic receptor antagonist, mecamylamine (Reid et al.,

1999; Zachariou et al., 2001), and by nicotinic receptor knock outs (Zachariou et al., 2001).

While nicotine-enhanced reinforcement has been well established in rodent models (Donny et al., 2003; Chaudri et al., 2006; Palmatier et al., 2006; Liu et al., 2007; Palmatier et al., 2007a, b; Caggiula et al., 2008), fewer studies have examined this phenomenon in humans. However, findings from the few published human studies are consistent with nonhuman literature. In one such study, Perkins and Karelitz (2013) examined nicotine's enhancement of positive reinforcers (like money and music) and negative reinforcers (like the termination of aversive noise). Similar to nonhuman findings, they determined that reinforced responses were significantly greater following the smoking of nicotinized cigarettes compared to denicotinized cigarettes or no smoking. Thus, findings from both human and nonhuman literature demonstrate that nicotine, acting as a reinforcement enhancer, magnifies the incentive value of accompanying stimuli, triggering drug-seeking and precipitating relapse (Abrams et al., 1998; Shiffman et al., 1996; Lesage et al., 2004; Ferguson & Shiffman et al., 2009).

1.2. Nicotine reward enhancement using the conditioned place preference paradigm

Most research to date has examined the effect of nicotine on reinforcement enhancement using operant behavioral paradigms. Recently, however, several studies have investigated whether nicotine's reinforcement enhancing effects extend to reward responsiveness using the conditioned place preference (CPP) paradigm. This traditionally nonhuman task measures the extent to which a rodent chooses to be in an environment that has been repeatedly paired with the appetitive effects of a stimulus (Tzschentke, 2007). Pavlovian conditioning is the most widely accepted explanation for CPP since it is believed that the context paired with the reward becomes a conditioned stimulus that predicts the presence of the reward. Advantages to CPP have been documented in a thorough review by Carr et al. (1989), indicating that the task is sensitive to low

drug doses, can be obtained in a single drug-pairing (Bardo, 2000), does not require a surgical procedure, and measures the effects of both reward and aversion. Furthermore, the test measurements are made in the absence of the drug; therefore, drug effects like motor impairment or stimulation do not confound the results. CPP can be induced by a number of natural rewards, including food, water, copulatory opportunity, and opportunity for social interaction, as well as by a variety of drugs (see review: Tzschentke, 2007). Specifically, nicotine-induced CPP in nonhumans has been well-documented by numerous studies (see reviews: Le Foll & Goldberg, 2005; Brielmaier et al., 2008).

To examine the effects of nicotine on reward responsiveness using CPP, Buffalari et al. (2014) demonstrated that a single, noncontingent injection of nicotine enhanced nonhuman place preference expression for contexts previously coupled with either sucrose or cocaine. Similarly, 7-day nicotine pre-treatment increased preference for an environment previously paired with cocaine (Levine et al., 2011). Nicotine also enhanced nonhuman conditioned place aversion to chambers previously paired with either lithium chloride or foot shock (Buffalari et al., 2016). Translating nonhuman findings of nicotine reward enhancement to humans, our lab previously demonstrated that nicotine administered to undergraduate participants as a 4mg lozenge prior to conditioning increased CPP expression for a virtual environment previously paired with chocolate food rewards (Palmisano et al., 2018). However, the results of this study were complex and may have been confounded by a nicotine dose that was too high for our nondependent sample of nicotine users. Therefore, while these studies demonstrate the efficacy of utilizing the CPP paradigm to the characterize reward mechanisms that underlie nicotine dependence, additional research is warranted.

1.3. Attribution of incentive salience to nicotine and non-drug cues

Addictive substances, like nicotine, and other positive reinforcers are believed to exert their rewarding effects by activating neurons within the ventral tegmental area (VTA) of the mesocorticolimbic reward pathway promoting the release of dopamine within the nucleus accumbens (NAc) and other brain regions (Corrigall et al., 1994). Therefore, dopamine stimulation is believed to play a central role in modulating appetitive behaviors, and dysregulation within these circuits may lead to nicotine dependence. Hedonic theories suggest that mesocorticolimbic dopamine mediates the pleasure of reward stimuli (Wise, 1988), since this system is activated by both natural and drug rewards, like food, sex, money, and nicotine (Kalivas, 2002; Kelley & Berridge, 2002; Di Chiara & Imperato, 1988). In fact, pleasurable ratings after smoking are significantly predictive of future use and smoking relapse (Strong et al., 2011). However, tolerance to the drug's euphoric effects frequently develops with chronic use, highlighting the limited role of hedonic pleasurability in nicotine dependence. Moreover, nicotine does not produce subjective euphoria in all nicotine users, suggesting a dissociation between hedonic pleasure and the excessive drive to consume drugs (Rose et al., 2000; Caggiula, et al. 2008).

Instead of mediating the subjective evaluation, or "liking," of rewards, others suggest that dopaminergic release mediates the motivational "wanting" to approach and consume rewards. Proposed by Robinson and Berridge (1993, 2000, 2001, 2008), the incentive-sensitization theory suggests that neuroadaptations resulting from chronic nicotine use sensitize neural circuits to excessively attribute incentive salience to drugs and drug-associated reward stimuli. In this sense, attentional processing is biased toward reward cues triggering excessive "wanting" for drugs, whether or not they are correspondingly "liked." Sensitized incentive salience can be

expressed as subjective craving or as physiological reactivity to reward cues, which have been implicated in the motivation to use nicotine (Shiffman, 1982; Niaura et al., 1988; Payne et al., 2006). For example, smokers subjectively report increased cravings (Wertz & Sayette, 2001; Sayette & Tiffany, 2013) and exhibit enhanced physiological responses (Geier et al., 2000) when exposed to drug cues, like a lit cigarette resting in an ashtray. However, literature is mixed regarding the magnitude of incentive salience devoted to nicotine rewards relative to alternate, non-drug reinforcers available to the user.

As mentioned, nicotine has been shown to increase behavioral responding for conditioned and unconditioned drug and non-drug reinforcers, indicating that nicotine enhances the reward value of these stimuli (Donny et al., 2003; Balfour, 2004; Chaudhri et al., 2006). Intracranial self-stimulation studies indicate that nicotine acutely lowers the threshold for self-stimulation, supporting the theory that nicotine-enhanced dopamine release increases neural sensitivity to rewards (Kenny & Markou, 2006). In this sense, any rewarding stimulus experienced during dopamine overflow would be imbued with enhanced motivational salience. However, limited research has been conducted to identify whether the degree of salience attribution differs between nicotine-related versus non-nicotine rewards leading one to question whether nicotine equally enhances the incentive value of drug and non-drug stimuli, or if enhanced motivational salience to nicotine cues modulates the reward value of non-drug cues.

Some research suggests that chronic nicotine use particularly enhances incentive salience of drug rewards and attenuates the attentional and motivational resources that remain for alternative cues (Franken, 2003). In other words, nicotine-dependent individuals may exhibit greater attention and approach toward drug-related cues at the expense of intrinsically-rewarding, non-nicotine stimuli (Blum et al., 2000; Goldstein and Volkow, 2002; Koob & Le Moal, 1997).

In support of this theory, several neuroimaging studies have identified elevated activity in brain reward regions to drug cues among nicotine abusers relative to healthy controls (David et al., 2005; Franklin et al., 2007). Contrarily, attenuated activity to non-drug rewards and positive hedonic stimuli have been reported in nicotine users relative to non-users (Martin-Soelch et al., 2003; Peters et al., 2011; Rose et al., 2012). This evidence suggests that nicotine users and non-users differentially assign motivational valence to rewards, where nicotine users may be more reactive to drug rewards than natural rewards.

Fewer studies have assessed motivational processing to both nicotine and non-nicotine rewards within the same paradigm, but some research supports the notion that drug users bias salience attribution to drug rewards and are hyposensitive to non-drug rewards. For example, neuroimaging reveals greater activation to smoking cues and hypoactivation to positive and negative affective stimuli in smokers, while non-smoker controls exhibit the opposite pattern (Diggs et al., 2013). Others have shown that nicotine-dependent individuals choose cigarettes over chocolate in a forced choice test, potentially demonstrating enhanced motivation for cigarettes and reduced motivation for chocolate (Hogarth 2012; Hogarth & Chase, 2011, 2012). Lawn et al. (2015) reported that dependent smokers explicitly chose cigarettes over both music and chocolate rewards but found that occasional smokers chose chocolate more than cigarettes. Nicotine abstinence, however, led to more choices for cigarettes and reduced preference for non-drug rewards in both groups. Interestingly, when evaluating reward processing using an operant conditioning task and a self-report measure of "liking" and "wanting," the same study reported that satiated dependent smokers exhibited no differences in reward processing for cigarettes, chocolate, or music. Satiated occasional smokers, on the other hand, pressed more for chocolate

than cigarettes and reported more “liking” and “wanting” for music than cigarettes (Lawn et al., 2015).

These results obfuscate the conclusion that nicotine biases incentive motivational processing toward drug cues at the expense of non-drug rewards; however, it does suggest that reward values may be polarized during periods of nicotine abstinence. Supporting this hypothesis, Freeman et al. (2012) used an associative learning task to show that abstinent smokers, but not satiated smokers or non-smoker controls, assigned higher reward value and had an attentional bias to smoking-related images compared to neutral cues predicting monetary rewards. Abstinence-induced activation of reward-related brain regions to smoking rewards and attenuated activation to monetary rewards has also been identified (Sweitzer et al., 2014).

Other studies, however, have found no evidence of reduced motivation for non-drug rewards at the expense of drug rewards. For example, Bühler et al. (2010) reported no effect of 36-hour abstinence on mesocorticolimbic activity to stimuli predicting monetary or cigarette rewards, nor were differences observed in the subsequent behavioral responses made to obtain these respective rewards in dependent and occasional smokers. In fact, in nondependent smokers, higher neural reactivity and instrumental responses rates were observed for monetary rewards relative to cigarette rewards (Bühler et al., 2010). Using acoustic startle reaction and facial electromyography, Geier et al. (2000) demonstrated that responses to smoking cues were comparable to those of pleasant control images, but significantly differed from unpleasant cues. This pattern of effects was similar in non-deprived, nicotine-deprived, and deprived smokers who expected to smoke. In never-smokers, however, physiological responses and EMG activity to smoking scenes matched those of unpleasant control images.

To summarize, while nicotine users and non-users exhibit different profiles of

motivational processing for rewards, few studies have evaluated salience attribution to nicotine and non-nicotine stimuli within the same model. Furthermore, findings from existing literature conflict and emphasize that the magnitude of incentive salience devoted to drug and non-drug rewards is incompletely understood. While nicotine-dependent individuals may attribute greater reward saliency to nicotine stimuli, it is unclear if this enhanced motivational processing modulates the reward value of non-drug cues.

1.4. The effects of nicotine on fear learning

Clearly, there is mounting evidence indicating that nicotine enhances the incentive motivational properties of cued and contextual stimuli via Pavlovian conditioning mechanisms, and it is believed that high levels of attributed incentive salience play a role in maintaining nicotine dependence and relapse after quitting. However, nicotine may also enhance the motivational value attributed to aversive cues, and it has been suggested that these aberrant Pavlovian conditioning mechanisms may be responsible for the development of anxiety disorders (AD), like post-traumatic stress disorder (PTSD; Morrow et al., 2011; Bush et al., 2007). In fact, changes in conditioned fear response magnitude have been proposed as a model for vulnerability to AD (Jovanovic et al., 2010; Yehuda et al., 2007; Guthrie et al., 2006).

Fear conditioning (FC) is a commonly used model to examine the encoding and stimulus-based retrieval of traumatic memories (Davis, 1992; Fendt & Fanselow, 1999; LeDoux, 2000; Maren, 2001). Although distinct AD classifications can be characterized by different symptoms, AD generally are distinguished by exaggerated fear responses to cues and contexts that are not dangerous (Shin & Liberzon, 2010). This suggests that by way of classical conditioning, learned anxiety resulting from a trauma or highly stressful period may be generalized to similar but neutral cues or contexts causing negative emotional responses, like fear and anxiety. To this

point, empirical evidence indicates that relative to healthy controls, AD patients display significantly greater conditioned fear responding following simple conditioning (see meta-analysis: Lissek et al., 2005). Moreover, studies suggest that fear and anxiety-related learning are specifically vulnerable to the effects of abused drugs with AD showing a particularly high rate of co-morbidity with nicotine dependence (Breslau et al., 2003; Feldner et al., 2007). Notably, the rates of nicotine dependence have been found to be significantly higher in individuals with AD (45.3%) compared to healthy individuals (22.5%; Ziedonis et al., 2008), and AD are more prevalent among individuals who smoke (22%) than in the non-smoking population (11.1%; Grant et al., 2006).

While the emotional responses elicited by cued and contextual conditioned stimuli are the same, the neurological pathways that mediate the two forms of FC are different. Learning to associate the context with the unconditioned stimulus is both hippocampus and amygdala dependent; whereas, forming an association between the cued conditioned stimulus and the unconditioned stimulus does not depend on the hippocampus, but does depend on the amygdala. Numerous nonhuman studies suggest that nicotine particularly facilitates hippocampal-dependent learning (Gilliam & Schlesinger, 1985; Grigoryan et al., 1996; Levin et al., 2002; Gould & Higgins, 2003). For example, acute nicotine enhances hippocampal-dependent contextual FC, but has no effect on hippocampal-independent cued FC (Davis et al., 2005, Davis et al., 2006a, b; Gould & Wehner, 1999, Gulick & Gould, 2008, Portugal et al., 2011). Interestingly, cued FC parameters can be modified to recruit the hippocampus if a temporal delay, during which no stimulus is presented, is inserted between the offset of the cue and the onset of the US (Solomon et al., 1986; Moyer et al., 1990; Büchel et al., 1999; McEchron et al., 1999; Crestani et al., 2002; Gould et al., 2004; Quinn et al., 2005). This type of hippocampal-dependent learning is called

trace fear conditioning and has been shown to be enhanced by nicotine (Gould et al., 2004; Raybuck & Gould, 2009; Davis et al., 2006b). To our knowledge, studies examining the effects of nicotine on learned fear have been exclusively limited to nonhumans; therefore, more research is needed to address whether nicotine also enhances fear learning in humans, with specific enhancement of hippocampal-dependent FC.

1.5. Dissertation Objectives

The overall objective of this dissertation research is to more clearly characterize the role of nicotine on human behavioral and physiological responding for both rewarding and aversive stimuli. Abundant preclinical evidence suggests that nicotine enhances reinforcement and reward-responsiveness (Chaudhri et al., 2006; Palmatier et al., 2006; Perkins & Karelitz, 2013; Palmisano et al., 2018). However, most studies to date have employed operant behavioral paradigms to investigate the reinforcement enhancing effects of nicotine instead of classical conditioning models like conditioned place preference (CPP; except see Buffalari et al., 2014). Moreover, our lab has solely attempted to translate these nonhuman studies to humans by examining the effects of a 4mg nicotine lozenge on CPP with complex findings possibly resulting from excessive nicotine dose in a nondependent sample of participants (Palmisano et al., 2018). Therefore, Study 1 of the present work assessed the ability of a lesser 2mg dose of nicotine to enhance human sensitivity to food rewards using our virtual reality conditioned place preference paradigm.

Additional research suggests that nicotine users and non-users differentially attribute motivational processing to rewards (David et al., 2005; Franklin et al., 2007; Martin-Soelch et al., 2003; Peters et al., 2011; Rose et al., 2012). However, while nicotine users may attribute greater reward saliency to nicotine stimuli, it is unclear if this enhanced motivational processing

modulates the reward value of non-drug cues (Diggs et al., 2013; Hogarth 2012; Hogarth & Chase, 2011, 2012; Lawn et al., 2015; Freeman et al., 2012; Bühler et al., 2010; Parker & Gilbert, 2008). Additional evidence suggests that individuals who score highly on characteristics related to impulsivity may exhibit enhanced incentive salience for nicotine and nicotine-associated stimuli (Perkins et al., 2000). Therefore, Study 2 explored the behavioral and physiological differences in reward responding between nicotine users and non-users and investigated the relationship between impulsive traits and reward processing. We also examined the effects of acute nicotine administration in abstinent nicotine users on these measures of reward processing.

Finally, nonhuman literature indicates that in addition to enhancing the incentive motivational properties of rewarding cues, nicotine may also enhance the motivational value attributed to aversive cues, where it has been suggested that these aberrant Pavlovian conditioning mechanisms may be responsible for the development of anxiety disorders (Morrow et al., 2011; Bush et al., 2007). Using a preclinical fear conditioning model, studies have demonstrated that nicotine particularly enhances hippocampal-mediated versions of fear learning, specifically contextual and trace fear conditioning, but not hippocampal-independent cued fear. These studies have been exclusively limited to nonhumans; therefore, Study 3 addressed whether nicotine also enhances fear learning in humans, with specific enhancement of hippocampal-dependent fear. We measured the effects of acute nicotine on cued and contextual conditioned fear using a novel virtual reality fear conditioning paradigm. Using a variation of this paradigm, we also examined the effects of acute nicotine on trace conditioned fear.

II. PARTIAL ENHANCEMENT OF HUMAN REWARD SENSITIVITY BY NICOTINE USING THE CONDITIONED PLACE PREFERENCE TASK

2.1. Introduction

The finding that nicotine enhances reinforcement has been replicated across a range of doses, routes of administration, schedules of reinforcement, and reinforcing stimuli, including conditioned reinforcers (Chaudhri et al., 2006; Palmatier et al., 2006; Olausson et al., 2004; Raiff & Dallery, 2006; Perkins & Karelitz, 2013). However, most studies investigating the reinforcement enhancing effects of nicotine employed operant behavioral paradigms, as opposed to classical conditioning models like conditioned place preference (CPP). Generally, the CPP task is comprised of two contextually distinct compartments joined by a connecting tunnel. The two compartments may differ across modalities such as visual, auditory, tactile and olfactory cues. During the task, the animal is confined to one of the two compartments for a fixed amount of time, and is given a rewarding substance, like food or drug. Later, in a separate session, the animal is confined to the other compartment and receives a placebo for an equal amount of time. These pairings are repeated to strengthen the association between context and presence or absence of the reward. Following the pairing sessions, a test session is given in which the animal receives unrestricted access to both compartments without any reward or placebo. Typically, animals demonstrate a strong preference for the room in which the reward was previously paired, despite the reward no longer being present (van der Kooy et al., 1987). Pavlovian conditioning is the most widely accepted explanation for the CPP since it is believed that the context paired with the reward becomes a conditioned stimulus that predicts the presence of the reward.

Only a few studies have utilized the CPP paradigm to extend nonhuman investigations of nicotine's reinforcement enhancing properties to reward responsiveness. Levine et al. (2011)

demonstrated that 7 days of nicotine pre-treatment facilitated place preference expression for a compartment previously paired with cocaine. Similarly, Buffalari and colleagues (2014) showed that nicotine respectively enhances sensitivity to cocaine and sucrose rewards. In this study, separate groups of rats were given either sucrose or cocaine in one of two CPP chambers for four consecutive days. Preference was then evaluated following a single injection of nicotine or saline. Consistent with prior studies examining nicotine's reinforcement enhancing effects, Buffalari et al. reported that nicotine enhanced preference for the context previously paired with either sucrose or cocaine. Notably, a significant effect was observed after a single nicotine injection, suggesting that CPP may be a more sensitive model than operant paradigms, which typically require several nicotine exposures to produce an effect (Caggiula et al., 2009; Chaudhri et al., 2006; Palmatier et al., 2012). The CPP paradigm also provides an innovative method to examine nicotine-enhancing effects on aversive stimuli where it has been shown that nicotine also enhances nonhuman conditioned place aversion to chambers previously paired with either lithium chloride or foot shock (Buffalari et al., 2016). Together, these studies demonstrate the efficacy of utilizing the CPP paradigm to characterize reward mechanisms that underlie nicotine dependence.

Given evidence that the reward-enhancing effects of nicotine extend to nonhuman CPP, our lab was of the first to attempt to translate these findings to humans. Using virtual reality to translate the standard CPP task to humans, we reliably established that humans also express CPP for food (Astur et al., 2014, 2015), virtual points (Astur et al., 2016) and monetary rewards (Childs, Astur, & de Wit, 2017). Most recently, we demonstrated that a single administration of a 4mg nicotine lozenge to undergraduate participants prior to conditioning increased CPP expression for a virtual environment previously paired with chocolate food rewards (Palmisano

et al., 2018). However, the results of this study were complex with the effect most strongly detected in nicotine-dependent participants who subjectively *rated* the previously rewarding room more favorably than the non-rewarding room. We did not see place preferences in nondependent participants, nor did nicotine-treated participants (regardless of dependence) spend more *time* in the rewarding context. It is possible that nondependent users, which comprised much of our undergraduate sample, are more sensitive to the aversive effects of this 4mg higher dose of nicotine, accounting for their lack of CPP. The 4mg lozenge was initially chosen as a dose and route of nicotine administration because it results in blood nicotine levels that are 4-fold higher than other smokeless nicotine products across a 1-hour timeline, with levels peaking approximately15minutes after administration (McEwen, et al., 2008; Palmisano et al., 2018). However, per pharmacokinetic investigations and manufacturer instructions, the 4mg lozenge is intended to address the needs of more highly dependent nicotine users who start smoking within 30 minutes after waking (Heatherton et al., 1989; Baker et al., 2007). Time to first cigarette is considered a simple but powerful index of nicotine dependence (Fagerstom, 2003; Baker et al., 2007) and is a suitable way of determining the nicotine "need" of each individual (Henningfield et al., 2009). In fact, some evidence suggests that the 4mg dose may actually be detrimental to smokers who are not highly nicotine dependent (Kornitzer et al., 1987). Thus, investigating CPP effects with a lower nicotine dose is warranted.

Using a lower dose of nicotine that may be more appropriate for a lesser-dependent sample of participants, the present study aimed to investigate the effect of a 2mg dose of nicotine on human reward sensitivity using our virtual CPP task. Given the robust reward-enhancing effects of nicotine in nonhumans, we hypothesized that participants who received a 2mg nicotine lozenge prior to conditioning would demonstrate enhanced CPP expression for a room

previously paired with chocolate food rewards compared to those who received placebo, as measured by both the amount of time spent in each room and the subjective ratings of how much they liked the room previously paired with chocolate reward.

We previously demonstrated that a single nicotine administration prior to training alone was sufficient in enhancing a ratings CPP relative to placebo (Palmisano et al., 2018), supporting our decision to administer nicotine prior to conditioning. Previous nonhuman studies have also found that nicotine pre-treatment injected subcutaneously prior to training produced a dose-dependent, specific increase in responding for the reinforcer or reward (Olausson et al., 2003, 2004a, b; Smith et al., 1999; Blomquist et al., 1996; Söderpalm et al., 2000). In one of few nonhuman studies to examine reward enhancement using place conditioning, Levine et al (2011) found that nicotine pre-treatment increased place preference expression for a cocaine-paired environment. In another study using a Pavlovian discriminatory approach task, rats were exposed to nicotine or saline for 15 consecutive days (Olausson et al., 2003). After three days of withdrawal, the water-deprived animals were trained on the task during which a compound light-and-tone cue was repeatedly paired with water. Prior to testing, animals received injections of either nicotine or saline. Importantly, no differences between pre-training or pre-testing nicotine treatment were observed, such that all nicotine-exposure regimes enhanced conditional responding (Olausson et al., 2003).

To elucidate the underlying motivational mechanisms that promote nicotine seeking and use, we also examined the relationship between CPP and several questionnaire metrics which assess various nicotine-related factors. These questionnaires included the Fagerstrom Test for Nicotine Dependence (FTND; Fagerstrom, 1989), the Questionnaire on Smoking Urges (QSU; Tiffany & Drobes, 1991), and the Behavioral Inhibition and Activation Scale (BIS/BAS; Carver

& White, 1994). The Fagerstrom Test for Nicotine Dependence is a standard instrument for assessing the intensity of physical dependence to nicotine. It contains six items that evaluate the quantity of cigarette consumption and the compulsion to use. Given our previous findings that some level of nicotine dependence was a prerequisite for nicotine-enhanced CPP (Palmisano et al., 2018), we hypothesized that those with higher indices of nicotine dependence would be more likely to demonstrate a CPP.

The Questionnaire on Smoking Urges is a 10-item self-report measure that measures the intensity of cravings since cravings are one of the most prominent symptoms of nicotine dependence and are a significant predictor of nicotine relapse. Neuroimaging studies indicate considerable overlap in the neural circuits underlying drug cue-induced craving and mesocorticolimbic activation involved in reward processing (Sinha, 2006). Therefore, this questionnaire was administered to explore mechanisms underlying reward-seeking behavior induced by nicotine-associated stimuli. We hypothesized that individuals who indicated greater craving reactivity would be more likely to express a CPP.

Finally, the Behavioral Inhibition and Activation Scale assesses the differences in the two motivational systems that likely contribute to cravings and relapse. The aversive motivational system, called the behavioral inhibition system (BIS), is hypothesized to control the experience of anxiety and to inhibit behavior that might produce negative or painful outcomes. Conversely, the behavioral activation system (BAS) is believed to induce positive affects like elation and happiness in response to cues of reward, and thus motivates goal-driven behavior toward cues. As mentioned, nicotine use often arises from the expectation that nicotine will alleviate negative affective states (Benowitz, 1996, 2009) and/or produce positive subjective sensations

(Henningfield et al., 1985). Therefore, we hypothesize that individuals who score highly on the subscales of these measures would be more likely to demonstrate a CPP.

2.2. Materials and Methods

2.2.1 Participants: Sixty-eight University of Connecticut undergraduates 18 years of age or older were recruited from introductory psychology classes. All participants were pre-existing nicotine users with varied levels of dependence; however, we did not differentially recruit high and low nicotine users. Nicotine-naïve participants were not used for the study as they are more likely to experience adverse side effects of nicotine administration. Participants were instructed to abstain from eating and from using nicotine for at least six hours prior to the experiment. Participants with cardiac conditions and those who were pregnant were ineligible to complete the study. Participants who were unwilling to eat chocolate during the study and those who subjectively rated themselves as experiencing high levels of nausea on the post-test survey were excluded post-hoc. Participants received class credit for their participation.

2.2.2. Apparatus: An IBM-compatible computer with a SVGA color monitor was used for testing. Participants seated at the computer navigated through the virtual environments by manipulating a joystick. Headphones connected to the computer were used to provide auditory feedback and a Med Associates Inc. ENV-203IR pellet dispenser was used to dispense M&Ms. into a tray for the participant to consume. Throughout the experiment, participant position within the virtual environment was written to a data file at 20 Hz.

2.2.3. Procedure: This was a one-day study in which participants were pseudo-randomly assigned to receive either a 2mg nicotine lozenge (GoodSense Polacrilex Lozenge, Mint) or similar-tasting placebo breath mint (Altoids, Wintergreen) prior to conditioning. A 2mg nicotine lozenge was chosen as the dose and route of nicotine administration because it results in stable

blood nicotine levels across a 1-hour timeline that are 8-10% higher in concentration than nicotine gum, with levels peaking approximately 15-minutes after administration (McEwen et al., 2008; Shiffman et al., 2005a). Moreover, the 2mg nicotine lozenge has been deemed suitable for lesser-dependent nicotine users (Shiffman, 2005b), which our previous study revealed largely makes up our undergraduate sample.

Participants arrived in the morning between 8:30 and 11:30 AM after at least six hours fasting and abstaining from nicotine use. Consent was obtained and tobacco abstinence was ensured using a CoVita Smokerlyzer carbon monoxide sensor. Participants with CO readings of PPM > 10 were rescheduled (Perkins et al., 2012). To note, CO readings would only be detected in individuals who smoke cigarettes or cigars, and not those who use other methods of nicotine consumption (i.e. e-cigarette, chewing tobacco, etc.). We did not have the means to record plasma nicotine levels to confirm use of other nicotine products. On a demographics pre-test questionnaire, participants completed questions regarding age, sex, when they last ate, when they last used nicotine, quantity and frequency of nicotine use, and methods of nicotine consumption. They were also asked to rate their level of hunger and their enjoyment of chocolate on a 1–10 scale (1 being "not at all," 10 being "extremely"). Participants were then administered either a 2mg nicotine lozenge (GoodSense Mini Polacrilex Lozenge, Mint) or placebo (Altoids, Wintergreen). Participants were instructed to place the lozenge in their mouth, occasionally moving it from side to side to allow it to slowly dissolve over the course of 15 minutes. They were told to minimize swallowing, and to not chew or swallow the lozenge. The same instructions were provided during placebo administration. Immediately following administration, participants completed several questionnaires including the Fagerstrom Test for Nicotine

Dependence, the Questionnaire on Smoking Urges, and the Behavioral Inhibition and Activation Scale.

Fifteen minutes after lozenge or placebo administration, participants underwent a 90-second practice session during which they were encouraged to explore a single, barren, never-to-be-seen-again virtual reality (VR) room using a joystick. To encourage exploration during all sessions of the experiment, participants were instructed to locate and collide with a downward facing arrow that appeared periodically in different locations within the virtual environment; however, there were no experimental consequences of these actions. Three M&Ms were dispensed at pseudo-random intervals during the practice session and participants were instructed to eat the M&Ms as they were dispensed.

After the practice session, each participant completed six, three-minute experimental acquisition sessions in a virtual environment. The following procedure and virtual environment were identical to those described in our previous study (Palmisano et al., 2018). The virtual environment contained two visually-distinct rooms connected by a neutral hallway (see Figure 1). During each of the six sessions, the participant was confined to one of the two rooms and was instructed to explore the environment with the joystick and to collide with the periodically-appearing downward facing arrows. One room was paired with real M&Ms for three sessions, and the other room was paired with no food for three sessions. The room paired with M&Ms and the orders of the pairing sessions were counterbalanced. During the M&M sessions, one M&M was periodically dispensed into a cup beside the participant, and the participant was instructed to eat the M&M as it was dispensed. Specifically, an M&M was dispensed every 21 s ± 5 s. Between 25 and 30 M&Ms in total were dispensed over the course of the experiment, which is approximately half the amount in a regular 47.9g single serving size bag of M&Ms.

Upon completing the six acquisition sessions, participants were given a 5-minute break before undergoing the three-minute test session. During the test session, participants began in the neutral hallway with access to the two conditioning rooms. After entering either conditioning room of their choice, access to the neutral hallway was blocked, but participants could move between the two rooms via a door between them. M&Ms were not dispensed during the test session. At the end of the test session, participants were electronically presented with a visual analogue scale asking them to rate their level of nausea on a 1–100 scale (0 being "not at all"). Participants were then given an electronic survey asking them how much they enjoyed each room on a scale of 0–100 (0 being "not at all"), and how much they enjoyed chocolate on a scale of 0–100 (0 being "not at all"). The virtual reality software recorded position coordinates and the amount of time spent and in each room during all sessions.

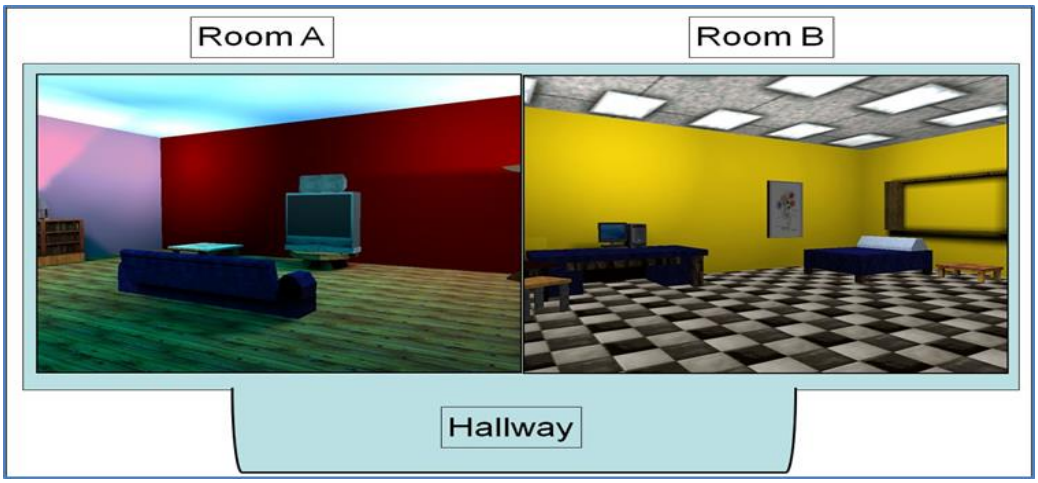


Figure 1. *Depiction of the virtual environment. Both rooms were identical in shape and size, but contained different items, colors and patterns.*

2.3. Results

2.3.1. Participants: Of the sixty-eight undergraduates recruited for this study, data from seventeen participants were excluded due to: equipment malfunction (n = 2), self-reported nausea (n = 6), or participant compliance error (i.e. incorrect lozenge administration or refusal to

eat M&Ms; n = 9). There was no statistical difference between the nicotine and placebo groups in attrition (n = 9 (26.5%) in the nicotine group and n = 8 (25.0%) in the placebo group; p > 0.05). Fifty-one participants were included in the final analysis ($n_{nicotine}$ = 25, 8 female, average age = 19.1 ± 0.95; $n_{placebo}$ = 26, 10 female, average age = 18.8 ± 1.26).

While all participants were required to be at least six hours abstinent prior to completing the study per inclusion criteria, on average, participants were 29.9 ± 28.4 hours abstinent with no statistical differences reported between treatment groups (F(1,49) = 2.34, p = 0.13). The preferred method of nicotine consumption was e-cigarettes, which was defined as any electronic nicotine delivery system (n = 36). Other methods of consumption included tobacco cigarettes (n = 13) and chewing tobacco (n = 2). On average, participants used approximately 15.7 ± 23.1 nicotine products weekly with nicotine-treated participants using significantly more products weekly than those who received placebo ($M_{nicotine}$ = 24.1 ± 30.0, $M_{placebo}$ = 7.5 ± 8.0; F(1, 49) = 7.41, p = 0.009). To ensure that this difference between groups did not influence the main effect of CPP, we ran an ANCOVA and found no effect of weekly nicotine products on time (F(1, 48) = 0.65, p = 0.43) nor ratings (F(1, 48) = 0.28, p = 0.60) difference scores as a function of treatment. Our current sample was largely comprised of light, intermittent nicotine users as demonstrated by an average Fagerstrom Test for Nicotine Dependence (FTND) score of 1.8 ± 1.58. A zero score indicates no dependence, a 1-5 score indicates low to moderate dependence, and anything greater than 5 indicates high dependence. There was no statistical difference between treatment groups in terms of nicotine dependence as measured by the FTND ($M_{nicotine}$ = 1.84 ± 1.62, $M_{placebo}$ = 1.76 ± 1.55; F(1, 49) = 0.03, p = 0.874).

2.3.2. Conditioned Place Preference: To examine our *a priori* hypothesis that each treatment group would display a CPP, we conducted paired-sample t-tests to determine whether

the amount of time spent in each room differed significantly from zero, indicating a CPP. We found that nicotine-treated participants demonstrated a CPP by spending significantly more time in the previously-paired M&M room compared to the unrewarded room during the test session (M_{MM} = 96.4 ± 30.2, M_{NoMM} = 70.4 ± 30.6; $t(24) = 2.15$, $p = 0.04$; Figure 2). However, the placebo group did not demonstrate a CPP in terms of time (M_{MM} = 89.1 ± 45.7, M_{NoMM} = 79.0 ± 44.2; $t(25) = 0.57$, $p = 0.57$; Figure 2). In terms of room ratings reported on a post-test questionnaire, neither the nicotine group (M_{MM} = 41.4 ± 33.0, M_{NoMM} = 48.6 ± 27.6; $t(24) = -1.14$, $p = 0.26$) nor the placebo group (M_{MM} = 43.8 ± 29.1, M_{NoMM} = 50.1 ± 29.1; $t(25) = -0.79$, $p = 0.44$) demonstrated a ratings place preference.

Conditioned place preference difference scores were then calculated by subtracting the amount of time spent in the non-M&M-paired room from the amount of time spent in the M&M-paired room during the test session, such that any score greater than zero indicated a CPP for the M&M-paired room. Difference scores in ratings of room liking were also calculated this way. A one-way ANOVA was conducted to assess whether the two treatment groups (nicotine vs. placebo) differed from one another in displaying a CPP. There was no significant difference between the groups in terms of CPP difference score time ($M_{nicotine}$ = 25.9 ± 60.3, $M_{placebo}$ = 10.0 ± 89.7; $F(1, 49) = 0.55$, $p = 0.46$). In other words, nicotine-treated participants did not spend significantly more time than placebo-treated participants in the room previously paired with M&Ms during the test session. A two-way ANOVA revealed no effect of participant sex on CPP difference score time ($F(1, 47) = 0.03$, $p = 0.86$), nor a sex x treatment interaction ($F(1, 47) =$

0.07, $p = 0.80$). In terms of CPP ratings, there was no significant difference between the treatment groups ($F(1, 49) = 0.01$, $p = 0.93$).

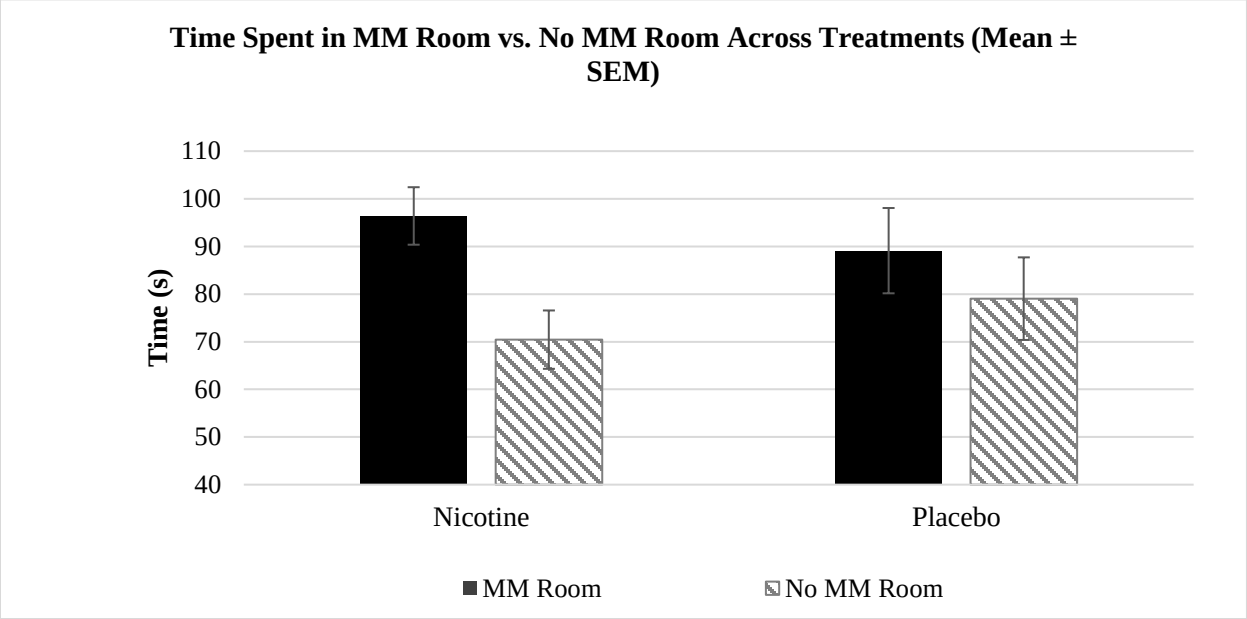


Figure 2. *The nicotine group displayed a CPP by spending significantly more time in the previously-paired M&M room during the test session compared to the No M&M Room (*$t(24) = 2.15$, $p = 0.04$*). The placebo group did not show a CPP (*$t(25) = 0.57$, $p = 0.57$*).*

2.3.3. Exploratory Analyses with Nicotine Dependence: As done in our preceding study examining the effect of nicotine on CPP (Palmisano et al., 2018), exploratory analyses were conducted to determine whether individuals with greater levels of nicotine dependence would be more likely to display a place preference. As mentioned, our current sample was largely comprised of light, intermittent nicotine users as demonstrated by an average Fagerstrom Test for Nicotine Dependence (FTND) score of 1.8 ± 1.58**.** Therefore, we conducted exploratory post hoc analyses using only individuals with nicotine dependence levels greater than 1 on the FTND questionnaire since we did not have large enough N's to examine those with higher dependence. This resulted in $n = 22$ (avg. age = 19.0 ± 1.25; nicotine products/week = 8.31 ± 7.37; avg. FTND = 3.22 ± 1.34) of which 10 received nicotine and 12 received placebo. For those with this

higher nicotine dependence, the treatment groups did not differ significantly in age or in the amount of nicotine products used per week.

In examining those with FTND > 1, we found that the nicotine group demonstrated a CPP by spending significantly more time in the previously-rewarding room (M_{MM} = 101.3 ± 33.2, M_{NoMM} = 64.7 ± 32.9; $t(9) = 1.76$, $p = 0.05$, one-tailed), but did not rate that room as significantly more enjoyable (M_{MM} = 45.9 ± 30.9, M_{NoMM} = 49.7 ± 28.6; $t(9) = -0.51$, $p = 0.62$). Nicotine-treated participants with FTND ≤ 1 did not show a significant CPP in terms of time (M_{MM} = 93.1 ± 28.7, M_{NoMM} = 74.3 ± 29.5; $t(14) = 1.27$, $p = 0.23$), suggesting that those with greater levels of nicotine dependence carried the CPP effect. Placebo-treated participants with FTND > 1 did not display a significant CPP in terms of time (M_{MM} = 102.3 ± 44.3, M_{NoMM} = 65.8 ± 41.1; $t(11) = 1.48$, $p = 0.17$) nor ratings (M_{MM} = 40.6 ± 26.7, M_{NoMM} = 57.8 ± 27.3; $t(11) = -1.87$, $p = 0.09$). Additionally, using difference scores, we found no significant difference between treatments in terms of the amount of time spent ($M_{nicotine}$ = 36.6 ± 65.7, $M_{placebo}$ = 36.4 ± 85.3; $F(1, 21) = 0.00$, $p = 0.99$), nor the ratings of each room ($M_{nicotine}$ = -3.8 ± 23.7, $M_{placebo}$ = -17.2 ± 31.8; $F(1, 22) = 1.21$, $p = 0.29$).

2.3.4. CPP and Questionnaire Metrics: Bivariate correlations were conducted to explore the relationship between CPP time and ratings difference scores and questionnaire metrics, including the Fagerstrom Test for Nicotine Dependence, the Questionnaire on Smoking Urges, and the Behavioral Inhibition and Activation Scale, as well as several VAS measures. Importantly, among nicotine-treated individuals, there was no correlation between CPP time difference score and "enjoyment of chocolate" rating ($r = 0.04$, $p = 0.86$) nor nausea ($r = 0.03$, $p = 0.89$), suggesting that the taste of nicotine and levels of nausea, respectively, did not influence the magnitude of M&M reward. There were no significant differences between treatments on any

of the questionnaire measures ($p > 0.05$), therefore, all other correlational analyses were collapsed across treatments. Interestingly, CPP ratings difference scores did not correlate with any questionnaire total measure nor subscale ($p > 0.05$). However, both the Urge to Smoke ($r = 0.36$, $p = 0.01$) and Relief of Negative Affect ($r = 0.43$, $p = 0.002$) subscales of the Questionnaire on Smoking Urges were significantly positively correlated with CPP *time* difference scores. In other words, individuals with a strong desire and intention to smoke with smoking perceived as rewarding, and those with the intention of smoking to relieve negative affect associated with withdrawal, were more likely to spend more time in the previously rewarding room. There was also a significant positive correlation between the Behavioral Activation Drive subscale of the Behavioral Inhibition and Activation Scale and CPP time difference scores ($r = 0.39$, $p = 0.004$). Behavioral activation is said to motivate goal-driven behavior toward positively reinforcing cues, while the Drive subscale particularly assesses behavioral responding.

Additionally, a trend was observed between the Fagerstrom Test for Nicotine Dependence and CPP time difference scores, such that those with higher levels of nicotine dependence were more likely to display a time CPP ($r = 0.26$, $p = 0.07$). Time difference scores did not correlate with the Behavioral Activation Fun Seeking ($r = 0.06$, $p = 0.68$) and Reward Responsiveness subscales ($r = 0.97$, $p = 0.61$), nor the Behavioral Inhibition subscale ($r = -0.15$, $p = 0.29$) of the Behavioral Inhibition and Activation Scale. The Fun Seeking subdomain measures the motivation to approach novel rewards while the Reward Responsiveness subscale measures sensitivity to positive reinforcers (Carver & White, 1994). The Inhibition subscale, on the other hand, evaluates the motivation to avoid aversive outcomes (Carver & White, 1994). Finally, there was no correlation between CPP time difference score and self-reported levels of nausea ($r = -0.01$, $p = 0.95$) , nor hunger across treatments ($r = -0.02$, $p = 0.89$). These null

findings suggest that nausea did not disrupt place conditioning and there was no influence of satiety over the course of the experiment.

2.4. Discussion

Given evidence of nicotine's reward-enhancing effects in nonhumans, we hypothesized that participants who received a 2mg nicotine lozenge prior to conditioning would demonstrate enhanced CPP during testing compared to those who received placebo. In the current study, we observed partial reward enhancement by nicotine in that nicotine-treated participants demonstrated a CPP by spending significantly more time in the previously-paired M&M room, while the placebo group did not demonstrate a CPP in terms of time nor ratings. However, we did not observe significant differences between treatment groups in terms of CPP time nor ratings difference scores. In other words, while the nicotine group demonstrated a significant CPP and the placebo group did not, the nicotine group did not spend significantly *more* time in the rewarded room than did the placebo group.

Findings of 2mg nicotine reward enhancement are consistent with our previous study which found that a 4mg nicotine lozenge also enhanced chocolate-reward conditioning using the human CPP paradigm (Palmisano et al., 2018). However, the 4mg effect was only detected in a subset of individuals with at least some level of nicotine dependence (FTND > 0). We originally postulated that nondependent individuals were perhaps more sensitive to the aversive effects of a higher 4mg dose of nicotine, perhaps explaining why nicotine's reward-enhancing effects were only evident in more dependent individuals. This supposition was supported by the present study where we observed that the 2mg nicotine group displayed a CPP regardless of nicotine dependence; however, those with at least some level of nicotine dependence (FTND > 1) again seemed to carry the CPP effect. Additionally, a trend was observed between the Fagerstrom Test

for Nicotine Dependence and CPP time difference scores, such that those with higher levels of nicotine dependence were more likely to display a time CPP. Given these findings, future studies should more intensively characterize the relationship between nicotine dependence and reward enhancement.

Contrary to findings in our previous study (Palmisano et al., 2018), the placebo group did not demonstrate a CPP in the current study. While it is unlikely, given the low levels of nicotine dependence reported by our sample (average FTND = 1.8 ± 1.58), we must still reflect upon the idea that individuals who received placebo were in withdrawal during the experiment, confounding the expected CPP. It is widely accepted that nicotine withdrawal is associated with a negative state consisting of both affective and somatic symptoms (Jackson et al., 2009). Moreover, elevated intracranial self-stimulation (ICSS) thresholds in nonhumans suggest that nicotine withdrawal decreases the sensitivity of brain reward systems, which can occur even in the absence of physical signs of withdrawal (Epping-Jordan et al., 1998; Watkins et al., 2000; Johnson et al., 2008). Behavioral assessments of motivation and effort for rewards also suggest that reward responsiveness is compromised during nicotine withdrawal in nonhumans (LeSage et al., 2006; Weaver et al., 2012; Pergadia et al., 2014) and humans (Al-Adawi & Powell, 1997; Dawkins et al., 2009; Pergadia et al., 2014). Therefore, it is possible that blunted reward sensitivity as a result of nicotine withdrawal may explain the lack of CPP in placebo treated individuals, particularly those with higher levels of nicotine dependence.

Interestingly, our correlational analyses revealed that individuals who used nicotine with the intention to relieve negative affect associated with withdrawal, were more likely to spend increased time in the previously rewarding room, further underscoring a potential role for nicotine withdrawal in CPP. Unfortunately, we did not have the foresight to collect a measure of

withdrawal symptoms; therefore, the influence of withdrawal on CPP must be addressed in future experiments. Nonetheless, our sample was comprised of light nicotine users who willingly abstained from nicotine use for longer than the required 6-hour window (average time since last use = 29.9 ± 28.4 hours) rendering the confounding influence of withdrawal effects from placebo treatment unlikely. Moreover, the withdrawal rationale cannot explain our previous observation in which the placebo group demonstrated a significant CPP by spending significantly more time in the previously-paired M&M room during the test day (Palmisano et al., 2018). An alternate explanation is the existence of paradigmatic differences between studies. Our previous study examined CPP over a 2-day period during which conditioning took place on Day 1 and testing occurred on Day 2. On the other hand, the current study occurred in a single day where testing took place 5-minutes after the final conditioning session since previous studies by our lab examining place conditioning using the present VR task produced successful CPP with the condensed paradigm (Astur et al., 2014; Astur et al, 2016)

Differences in the memory consolidation mechanisms that underly CPP may have resulted from these experimental variations accounting for the discrepant results across studies, particularly because nonhuman data suggests that activation of signaling pathways downstream from nicotinic acetylcholine receptors are essential for conditioned responding. For example, several studies using multiple-day conditioning paradigms have shown that phosphorylation of the CREB transcription factor is necessary to produce a nicotine CPP (Brunzell et al. 2009; Pluzarev & Pandey 2004; Walters et al. 2005). Additionally, disruption of CREB activation using HSV-mediated gene transfer (Brunzell et al., 2009) or mecamylamine (Pascual et al., 2009) prior to training blocked nicotine place preference. Again, the findings of these studies resulted from multiple-day conditioning paradigms while our study was conducted in one day.

The timeline of these mechanisms remains to be investigated humans; therefore, replication of studies is needed to know how consolidation influences human conditioned responding.

Interestingly, while the nicotine group demonstrated a significant CPP and the placebo group did not, we did not observe a significant difference between treatment groups. In other words, nicotine-treated participants did not spend significantly more time in the previously rewarding room than those who received placebo. This finding somewhat complicates the interpretation that nicotine enhances reward sensitivity in humans. While our lab (Palmisano et al., 2018) and several others (Olausson et al., 2003, 2004a, b; Smith et al., 1999; Blomquist et al., 1996; Söderpalm et al., 2000) have demonstrated that nicotine administered prior to training alone enhances responding for a reinforcer or reward, others have failed to find this effect. For example, while four consecutive days of nicotine pre-treatment in adolescent rats significantly enhanced cocaine-reinforced responding compared to saline controls or adults, this nicotine pre-treatment effect did not increase responding for sucrose pellets in adolescents. These results suggest that the reinforcement enhancing effect of nicotine administered pre-training does not generalize to all reward types, and even differs across stages of development (McQuown et al., 2007). Bridging findings between operant and conditioning paradigms, Levine et al. (2011) used the CPP procedure and found evidence of nicotine-enhanced cocaine reward responsiveness, demonstrating that 7 days of nicotine pre-treatment enhanced cocaine place conditioning. Conversely, Kelley & Middaugh (1999) reported that five weeks of chronic nicotine pre-treatment produced a conditioned place aversion to a previously-cocaine-paired context during nicotine-free testing. In these studies, nicotine administration pre-conditioning was administered chronically, instead of as a single pre-conditioning nicotine administration, making comparisons between these studies and the present study challenging.

More closely approximating the conditions of the present study, Grieder et al. (2009) used the CPP procedure to examine whether acute nicotine administration timing plays a role in nicotine reward sensitivity in nondependent mice. They reported that mice elicited a conditioned place aversion to a nicotine-paired environment when they were tested immediately after a single dose of nicotine pre-conditioning. However, a preference for the nicotine-paired environment was observed when mice were tested 8 hours after acute nicotine administration, and no motivational effect was observed when animals were tested 4 and 12 hours after treatment. These results suggest that reward responsiveness may differ as an effect of nicotine administration timing. Furthermore, in the only study to use the CPP procedure to demonstrate that a single nicotine injection enhances both cocaine and sucrose place conditioning, respectively, Buffalari et al. (2014) administered nicotine after conditioning and prior to testing. Combined, these results suggest that paradigmatic differences between preclinical literature and the present study may account for our inability to demonstrate nicotine-enhanced conditioning. Future studies should investigate whether acute nicotine administered prior to both conditioning and testing, or prior to testing alone, is sufficient to garner enhanced conditioning. Moreover, differences between a single nicotine administration and chronic nicotine pre-treatment should also be investigated.

Other methodological differences between existing nonhuman studies and the present study must also be considered. The majority of studies reporting nicotine-induced CPP in nonhumans have used a biased Pavlovian conditioning procedure (see review: LeFoll & Goldberg, 2005). In the biased design, animals receive pre-exposure to both contexts to establish context preference, and nicotine is then repeatedly administered in the initially non-preferred environment. To demonstrate that nicotine enhances both sucrose and cocaine CPP, Buffalari et

al. (2014) utilized a biased design. Levine et al. (2011), however, produced nicotine CPP enhancement for a cocaine-paired context using an unbiased design. The present study also utilized an unbiased design since the interpretation of CPP data using a biased procedure can be problematic, as it may reflect a preference shift due to a reduction of aversion as opposed to enhancement by reward (Torrella et al., 2004); however, future studies should investigate whether these paradigmatic differences influence the results.

Of course, pharmacokinetic differences between human and nonhuman studies must also be addressed as a potential explanation of study discrepancies. The seminal nonhuman studies investigating the effects of nicotine on operant responding for nonpharmacological stimuli often administer nicotine repeatedly via osmotic pump infusions (Donny et al., 1999, 2003; Caggiula et al., 2001; Palmatier et al., 2005; Chaudhri et al., 2006). This route of administration allows for rapid effects, and precise and reproducible dosing. However, the nicotine doses used in these prior studies generally exceed nicotine concentrations typically experienced by a smoker (Sofuoglu & Mooney, 2009; Benowitz & Jacob, 1990); therefore, nicotine infusions may not fully mimic nicotine delivered by inhalation by smoking, nor the effects of nicotine delivered by lozenge, as in the present study. While nicotine lozenges are unobtrusive and easy to administer, they do not produce the same degree of drug "liking" that intravenous or osmotic infusions do (Harvey et al., 2004; Houtsmuller et al., 2003; Sofuoglu & Mooney, 2009). Moreover, dose response curves between the routes of nicotine delivery differ considerably, where the stimulatory and pleasurable effects of intravenous nicotine peak approximately 1 minute post-infusion (Mello et al. 2013; Jensen et al, 2016), while 2mg nicotine lozenge effects peak approximately 15-20 minutes post administration (McEwen et al., 2008; Choi et al., 2003). Moreover, nonhuman studies benefit from highly controlled experimental settings where animal

weight, nicotine dose, and timing of delivery are less variable than in human studies. Therefore, these differences may complicate the detection of nicotine's effect on reward enhancement.

Several studies examining various routes of nicotine delivery on human reinforcement enhancement further suggest that the specificity of reinforced responding may be dependent on the route and dose of nicotine delivery. In a series of studies by Perkins and colleagues, reinforced responding was assessed using the "Apple Picker" operant computer task (Norman & Jongerius, 1985) during which participants had to search for "apples" by moving a cursor on the computer monitor and clicking the joystick when the cursor landed on a "tree." Participants received visual feedback when an apple was found, indicating that a unit of reinforcer had been earned for that trial. Each session consisted of four trials where each trial differed in the type of reward available (money, preferred music, termination of aversive noise, or no reward). A progressive ratio reinforcement schedule was used, and participants were free to stop responding at any point and wait for the end of the 15-minute trial period.

Using this task to examine the effect of nicotine e-cigarettes on reinforced responding relative to placebo e-cigarettes, Perkins et al. (2015) reported reinforcement enhancement for video rewards in nicotine-dependent participants, but no enhanced responding for any other reward types, like listening to preferred music. Similarly, compared to placebo, reinforced responding due to nicotine via spray or patch was greater for video reward, but not for music reward (Perkins et al., 2018). In nondependent subjects, however, nicotine via nasal spray had no influence on the reinforcing value of monetary or music rewards, nor the termination of aversive noise (Perkins et al., 2009). These results suggests that reinforcement enhancement varies as a function of the route of nicotine delivery and may interact with the degree of nicotine dependence.

Prior human studies using this operant paradigm also suggest that nicotine does not globally enhance reinforcement in humans, but instead may only enhance reinforcement for certain types of rewards. For example, using a within-subjects design, both nondependent and nicotine-dependent individuals participated in three experimental sessions of the aforementioned "Apple Picker" task following overnight abstinence. Sessions involved no smoking, smoking denicotinized cigarettes, or smoking nicotine cigarettes. Acute nicotine intake from smoking enhanced the reinforcing value of music rewards, but not any other reward type. Moreover, this reinforcement enhancing effect did not differ between dependent and nondependent smokers (Perkins & Karelitz, 2013). Another study by the same group demonstrated that nicotine via cigarettes increased responding for video rewards (Perkins et al., 2014), and that nicotine from a full cigarette, but not by a half cigarette or less, enhanced reinforced responding for highly preferred music rewards, but not moderately or less preferred music rewards (Perkins & Karelitz, 2013). These clinical studies suggest that nicotine's reinforcement enhancing effects are specific to certain types of rewards, particularly those that are sensory in nature.

In sum, the ability of nicotine to acutely increase responding may depend on reward type, as well as the dose and route of nicotine delivery. It is important to note that these studies exclusively employed operant behavioral paradigms. Therefore, additional research is needed to examine the effects of reward type and alternate methods of nicotine consumption on reward enhancement using the CPP procedure and other classical paradigms. It is possible that nicotine's reward enhancing effects may be more prominent under conditions different than those of the present study.

In terms of limitations, we did not have the financial means to record plasma nicotine levels to confirm dose and speed of nicotine delivery across participants. Plasma nicotine levels

would allow us to more intimately detect nicotine abstinence adherence prior to the study. The present study used CO readings to ascertain abstinence, a measure that is only sensitive to nicotine products containing tobacco. Given that a majority of our sample reported (tobaccoless) electronic nicotine delivery as their primary method of use, plasma nicotine readings are important for future studies. We also did not have the foresight to collect data on nicotine withdrawal symptom severity. Given the role of nicotine withdrawal in moderating reward sensitivity, an index of withdrawal symptoms is critical for future studies. Additionally, confirmation of these findings requires evaluation of reward type specificity and alternate methods of nicotine consumption as potential influencing factors on nicotine reward enhancement. It is possible that the pharmacokinetic properties of the 2mg nicotine lozenge attenuate reward enhancement relative to other routes of nicotine delivery, like intravenous routes typically used in nonhuman studies. Moreover, nicotine reward enhancement may also be more pronounced for other types of rewards, like those sensory in nature. Future studies should also selectively recruit groups based on nicotine dependence levels, since results suggest that dependence severity may predict differential responses to conditioning.

The present findings extend previous research by examining the influence of nicotine on human reward enhancement using the CPP paradigm. Combined with our previous study, the results suggest that nicotine at least partially enhances conditioning for chocolate food rewards in humans since participants who received 2mg nicotine demonstrate a significant CPP, while those who received placebo do not. These novel findings have important relevance to the field of substance abuse as they pertain to how Pavlovian conditioning factors generalize to humans. Importantly, determining the conditions by which nicotine enhances reward will increase our understanding of its ability to maintain nicotine use and will allow us to better design

intervention and treatment plans aimed at minimizing drug cravings, use, and relapse.

III. BEHAVIORAL AND PHYSIOLOGICAL DIFFERENCES IN REWARD PROCESSING BETWEEN NICOTINE USERS AND NON-USERS AND THE EFFECT OF ACUTE NICOTINE ADMINISTRATION

3.1. Introduction

It has been suggested that repeated exposure to nicotine may sensitize dopaminergic reward circuitry to excessively attribute incentive salience to otherwise neutral stimuli, a psychological process involved in appetitive behavior (Robinson & Berridge, 1993, 2000, 2001, 2008). Abundant evidence indicates that nicotine users and non-users differentially assign motivational valence to nicotine rewards. Relative to non-users, neuroimaging studies show elevated activity in brain reward regions to drug cues among nicotine users (David et al., 2005; Franklin et al., 2007). Moreover, behavioral studies suggest that smoking-related images are rated as subjectively more pleasant by nicotine users compared to non-users (Mucha et al., 1999; Mogg et al., 2003; Bradley et al., 2004, 2008). Nicotine users are also relatively faster than non-users to categorize smoking-related stimuli if the appropriate response is to approach the pictures rather than move away from them (Mogg et al., 2003; Bradley et al., 2004).

In addition to demonstrating hypersensitivity to nicotine rewards, several studies report that relative to non-users, nicotine users exhibit attenuated neural activity to non-drug rewards and positive hedonic stimuli (Martin-Soelch et al., 2003; Peters et al., 2011; Rose et al., 2012). Some posit that diminished response to non-drug rewards reflects inherent hypodopaminergic activity in the brain reward system, predisposing an individual to seek nicotine and engage in other behaviors to release dopamine to overcome such deficits (Blum et al., 2012; Anselme et al, 2009). Others suggest that hyporeactivity to non-drug rewards is acquired after chronic nicotine use, which particularly enhances the incentive salience of drug rewards and attenuates the

attentional and motivational resources that remain for alternative cues (Franken, 2003). Relatively few studies have assessed motivational processing to both nicotine and non-nicotine rewards within the same paradigm. One study used functional magnetic resonance imaging to measure blood oxygen level dependent response to smoking-related images, as well as affectively-positive erotic images and affectively-negative mutilation images (Diggs et al., 2013). They found that non-smokers demonstrated greater reactivity to both types of emotional images relative to smoking images. However, satiated smokers exhibited greater activation to smoking images and decreased activation to both positive and negative affective images.

Another study used a computer-based choice task to index dependent and occasional smoker preference for cigarette and non-drug rewards after 12-hour nicotine abstinence or ad libitum smoking (Lawn et al., 2015). During each trial, two cues were displayed on the screen representing the type of reward available. Rewards included cigarettes, music, chocolate, or a paper control. After making their choice, participants could then work for the delivery of a real version of their chosen reward in a fixed ratio schedule by pressing the spacebar on the keyboard. Compared to occasional smokers, dependent smokers made more choices for and more presses for the cigarette reward, an effect that was magnified by nicotine abstinence. No differences in instrumental responding for cigarettes, music, and chocolate were found within the dependent user group; however, they did choose cigarettes more than any other non-drug reward type during the forced choice task. Interestingly, occasional nicotine users made more choices and more presses for chocolate than cigarettes. In addition to examining the motivational salience attributed to drug and non-drug rewards, this study was also the only study to our knowledge to include a self-report measure of reward "liking." No differences were observed in the hedonic ratings of cigarettes, music, or chocolate among dependent users. However, within

the occasional group, music was rated as more pleasurable than cigarettes (Lawn et al., 2015). Therefore, the hedonic "liking" of rewards seems to be psychologically dissociable from motivated "wanting," and must also be investigated to understand how these systems work together to process reward.

Combined, these studies support theories that nicotine users demonstrate enhanced incentive salience for nicotine rewards. However, it is possible that different profiles of reward sensitivity exist between those with differing levels of nicotine dependence, and nicotine abstinence may also play a role in moderating salience attribution. The results from Diggs et al. (2013) and others (Hogarth, 2012; Hogarth & Chase, 2011, 2012; Lawn et al., 2015; Freeman et al., 2012; Sweitzer et al., 2014) support the notion that nicotine users not only bias salience attribution to drug rewards but are hyposensitive to non-drug rewards. However, Lawn et al. (2015) found little evidence of non-nicotine reward hyposensitivity. Other studies have also failed to show evidence of reduced motivation for non-drug rewards among nicotine users (Bühler et al., 2010; Geier et al., 2000; Epstein et al., 1991).

Parker & Gilbert (2008) used an EEG measure called stimulus preceding negativity (SPN) to examine smoker and non-smoker reactivity to nicotine cues, as well as affectively-positive and neutral non-drug images. SPN is a measure of brain activity that occurs during the anticipation of emotionally-significant stimuli and has been linked to neural mechanisms associated with arousal and motivational salience (Takeuchi et al., 2005; Lang et al., 2005; Parker & Gilbert, 2008). During the study, participants were presented with fixation cross-hairs on a computer screen. The cross-hairs were then replaced with a picture, before being replaced again with cross-hairs. The same picture was then presented a second time for a longer duration. Therefore, after the first image presentation, participants knew which picture would be presented

a second time. In non-smokers, mean SPNs were significantly greater for affectively-positive and neutral pictures than for nicotine-related pictures. On the other hand, mean SPNs of smokers were significantly greater for nicotine-related and positive images than neutral images. However, no differences were found between the SPNs for nicotine and positive images (Parker & Gilbert, 2008), indicating that smokers may express equal motivational processing for nicotine and non-nicotine rewards.

This finding supports evidence demonstrating that nicotine enhances the incentive value of non-drug rewards (Donny et al., 2003; Balfour, 2004; Chaudhri et al., 2006). For example, nicotine administration has been shown to increase reinforced responding for food and food-associated conditioned stimuli in nonhumans (Popke et al., 2000; Wing & Shoaib, 2010; Grimm et al., 2012; Raiff & Dallery, 2006). Moreover, nicotine has been shown to potentiate the reinforcing properties of rewarding stimuli in humans. Social factors have been shown to maintain nicotine use (Moran et al., 2004) and influence rates of nicotine abstinence (Cengelli et al., 2012). Some of these effects may occur due to the direct effects of nicotine on the perception of social stimuli. In a novel study, Attwood and colleagues (2009) assessed the effects of acute nicotine on ratings of facial attractiveness since facial attractiveness is correlated with activation of the mesocorticolimbic reward pathway (Aharon et al., 2001; Kampe et al., 2001; O'Doherty et al., 2003). Therefore, the magnitude of pleasurable responses to these stimuli may reflect the degree to which incentive-salience is being activated. After 24-hour nicotine abstinence, nondependent smokers were randomly assigned to receive either a nicotine or denicotinized cigarette. After smoking, participants were presented with stimuli of male and female faces on a computer screen and were asked to rate the images on a 7-point Likert scale. Individuals in the nicotine condition rated images as significantly more attractive than did those who received

placebo. Moreover, they observed no treatment effects on subjective ratings of mood, suggesting that the effects of nicotine on attractiveness ratings were not a result of global hedonic enhancement (Attwood et al., 2009). Therefore, this paradigm provides an innovative method to examine nicotine's ability to enhance the positively reinforcing properties of non-drug, social rewards in humans.

Summary: To summarize, abundant evidence suggests differential reward processing between nicotine users and non-users where nicotine users seem to excessively attribute incentive salience to nicotine-associated stimuli relative to non-users. However, it is unclear if this enhanced motivational processing for drug rewards modulates the reward value of non-drug cues since few studies have evaluated salience attribution to nicotine and non-nicotine stimuli within the same model. Moreover, findings from the existing literature conflict. Some suggest that hyperreactivity to drug rewards among nicotine users reduces the attentional and motivational resources that remain for non-drug cues. Others have observed equally enhanced salience attribution to nicotine and non-nicotine rewards. These discrepant findings lead one to question the direction of change in the processing of non-nicotine rewards among nicotine users, if any. A meta-analysis of drug cue reactivity literature (Carter & Tiffany, 1999) revealed that incentive motivation was reliably detected using physiological measures of skin conductance, however no studies to our knowledge have utilized skin conductance responses to investigate the effects of nicotine on drug and non-drug salience attribution. Additionally, only one study (Lawn et al., 2015) simultaneously evaluated self-reported "liking" and motivated "wanting" of drug and non-drug rewards. Compared to nondependent individuals who have had some nicotine exposure, dependent smokers retrospectively report having had greater positive sensations the first time they smoked (Hu et al. 2006; O'Connor et al. 2005; Pomerleau et al. 2004). Since

hedonic responses to nicotine may predict future use (Pomerleau et al., 1993; Strong et al., 2011; Zabor et al., 2012), it is clinically relevant to also assess the potential influence of "liking" on nicotine and non-nicotine reward processing.

Interestingly, enhanced pleasurable effects of nicotine have been reported among individuals who score highly on characteristics related to impulsivity, including disinhibition and sensation seeking (Perkins et al., 2000), suggesting that impulsive individuals may be more sensitive to the rewarding and reinforcing effects of nicotine. Consistent with this idea, studies show that nicotine users are more affected by delays to reinforcement than non-users (Sweitzer et al., 2008; Baker et al., 2003; Bickel et al., 1999, 2008; Mitchell, 1999, 2004; Wilson et al., 2015). Delays to reinforcement are often quantified using the delay discount procedure in which an individual must choose between a smaller-immediate reward or a larger-delayed reward. Impaired discounting has been correlated with indices of impulsivity (Green & Myerson, 2004), which includes features of risk-taking (de Wit et al., 2007). Therefore, it is perhaps unsurprising that nicotine users also demonstrate riskier decision-making than non-users on a computerized measure of risk-taking called the Balloon Analogue Risk Task (BART; Lejuez et al., 2002, 2003). Given that smoking onset (Burke et al., 2001, Burt et al., 2000; Harakeh et al., 2006; Masse & Tremblay, 1997) and relapse rates (Krishnan-Sarin et al., 2007; Diergaarde et al., 2008) are associated with impulsive personality characteristics, it is critical to investigate the relationship between impulsive traits and reward processing to inform etiological models and treatment interventions.

The present study employed three behavioral tasks and a battery of questionnaires to better characterize the role of nicotine in the assignment of motivational valence to nicotine and non-nicotine rewards, and to identify potential correlates between impulsivity and reward

responding. The Balloon Analogue Risk task (BART) was used to assess differences in risk-taking between nicotine users and non-users where we hypothesized that nicotine users would make riskier choices than non-users. To assess whether nicotine users attribute enhanced incentive salience to non-drug rewards relative to non-users, participants were asked to rate the attractiveness of a series of standardized facial stimuli. We hypothesized that relative to non-users, nicotine users would rate facial images as significantly more attractive. Finally, we employed a second image-rating task designed to evaluate salience attribution to nicotine and non-nicotine rewards within the same model. We hypothesized that the presentation of nicotine-associated stimuli would result in higher skin conductance responses and enhanced ratings of subjective valence and arousal among nicotine users relative to non-users. We also hypothesized that we would observe greater physiological responses to and higher ratings of nicotine-associated stimuli relative to neutral and negative affective imagery within the nicotine user group. However, given discrepancies in the literature, we made no predictions regarding the direction of responses assigned to emotionally-positive non-drug stimuli relative to nicotine images among nicotine users. In a serially-conducted experiment, we administered either nicotine or placebo to a separate group of nicotine-experienced participants to assess the influence of acute nicotine administration on these tasks. We hypothesized that relative to placebo, nicotine would magnify the expected behavioral and physiological outcomes.

3.2. Materials and Methods

3.2.1. Participants: Two hundred and thirty-seven University of Connecticut undergraduates 18 years of age or older were recruited from introductory psychology classes. Data were collected from both nicotine non-users and pre-existing nicotine users with varied levels of dependence; however, we did not differentially recruit high and low nicotine users.

Nicotine non-users were defined as individuals who used one or fewer nicotine products per month. Nicotine users were defined as individuals who used at least one nicotine product per week. Participants were instructed to abstain from using nicotine for at least six hours prior to the experiment. Participants with cardiac conditions and those who were pregnant were ineligible to complete the study.

3.2.2. Apparatus: A 17-inch IBM-compatible computer with a SVGA color monitor was used for testing. Sounds were presented at 90dB through headphones. Physiological measurements were recorded using a Biopac Systems MP150 data acquisition system. The Biopac MP150 system was connected via an Ethernet cord to a laptop running Biopac Acqknowledge software, version 4.2.1. The Biopac MP150 system received digital TTL signals through its isolated digital interface connecting to the parallel port on the stimulus computer running the VR software E-prime. Electrodermal activity (EDA) was collected continuously from two disposable electrodes on the index and middle fingers on the participant's non-dominant hand.

3.2.3. Procedure: Upon participant arrival, consent was obtained, and nicotine abstinence was ensured using a CoVita Smokerlyzer carbon monoxide sensor. Participants with CO readings of PPM > 10 were rescheduled (Perkins et al., 2012). On a demographics pre-test questionnaire, participants completed questions regarding age, sex, when they last ate, when they last used nicotine, quantity and frequency of nicotine use, and methods of nicotine consumption. Based on demographic survey responses, participants identified as current nicotine users who used at least one nicotine product weekly were randomly selected to receive either a 2mg nicotine lozenge (GoodSense Mini Polacrilex Lozenge, Mint), similar-tasting placebo breath mint (Altoids, Wintergreen), or nothing at all (see section *2.3.3. Procedure* for dose rationale and

administration details). Nicotine non-users who consumed less than one nicotine product per month were recruited as untreated controls.

Following treatment administration, all participants were asked to complete the following questionnaires in random order: the Fagerstrom Test for Nicotine Dependence, the Positive and Negative Affective Schedule, the Behavioral Inhibition and Activation Scale, the Sensitivity to Punishment and Sensitivity to Reward Questionnaire, the Barratt Impulsiveness Scale, the Zuckerman Sensation Seeking Scale, and the Minnesota Nicotine Withdrawal Scale. The Fagerstrom Test for Nicotine Dependence (FTND; Fagerstrom, 1989) is 6-item questionnaire used to measure the quantity of nicotine consumption and the compulsion to use. The Positive and Negative Affective Schedule (PANAS; Watson et al., 1988) is a 20-item self-report measure of positive and negative emotional states. The Behavioral Inhibition and Activation Scale (BIS/BAS; Carver & White, 1994) is a 24-item questionnaire used to measure individual differences in reward and punishment sensitivity by assessing the inhibition and activation motivational systems that likely contribute to drug-seeking and use. The Sensitivity to Punishment and Sensitivity to Reward Questionnaire (SPSRQ; Torrubia et al., 2001) is a 48-item self-report measure with "yes" or "no" questions related to specific cues and situations. Responses are divided into two subscales: Sensitivity to Reward (SR) and Sensitivity to Punishment (SP), developed to test behavioral inhibition and activation systems. The Reward Responsiveness Scale (RR; Van den Berg et al., 2010) is an 8-item measure of responsivity to reward. The Barratt Impulsiveness Scale (Patton et al., 1995) is the most widely used, 30-item self-report designed to assess the behavioral construct of impulsiveness. Another measure of impulsive personality characteristics, the Zuckerman Sensation Seeking Scale (SSS-V; Zuckerman, 2007) is a 40-item forced-choice questionnaire used to assess individual differences

in sensory stimulation and arousal. Subscales include thrill and adventure seeking, experience seeking, disinhibition, and boredom susceptibility. Finally, the Minnesota Nicotine Withdrawal Scale (MNWS; Cappelleri et al., 2005) is a 15-item scale that scores withdrawal symptoms listed in the Diagnostic and Statistical Manual of Mental Disorders (DSM–V; American Psychiatric Association, 2013) on an ordinal scale ranging from 0 (not present) to 4 (severe).

3.2.3.1 Balloon Analogue Risk Task: After questionnaire completion, all participants underwent the Balloon Analogue Risk Task (BART; Lejuez et al., 2002). This computerized measure of risk-taking behavior assesses individual differences in balancing the potential for reward versus loss. During the task, participants seated at a computer were presented with an image of a virtual balloon accompanied by a balloon pump. Each click of the pump caused the balloon to inflate and provided the participant with a $0.25 virtual reward. However, each balloon had an unspecified explosion point, such that one balloon might explode after ten clicks, while another might explode after thirty clicks. If the balloon exploded, virtual earnings for that trial were lost. At any point during the trial, participants could choose to stop pumping the balloon and "collect" their virtual rewards. The explosion of a balloon or the collection of virtual rewards signified the end of the trial and a new balloon would appear until 10 total balloon trials had been completed. A total of 10 balloons was chosen since identical results have been found using 10 balloons or the task maximum of 30 balloons (Lejuez et al., 2003). Participants were aware that their earnings were virtual, and that they would not receive real monetary earnings upon completing the task. However, in an exploratory derivative of the BART task, a subset of participants was informed they would be entered into a lottery for a real $20 Amazon gift card based on their BART task performance. One entry was awarded for every $0.25 virtual cents collected. This modification was employed to assess whether real earnings relative to virtual

earnings altered reward saliency and affected participant responses.

3.2.3.2. Facial Attractiveness Test: Following completion of the BART task, participants underwent the Facial Attractiveness Test during which they were asked to subjectively rate the attractiveness of a standardized set of male and female facial stimuli. Given that facial attractiveness is correlated with activity in certain reward pathways (Aharon et al., 2001; Attwood et al., 2009), the magnitude of pleasurable responses to these stimuli may reflect the degree to which incentive-salience is being activated. Facial cues consisted of sixty photographic facial stimuli (30 male, 30 female; 10 attractive, 10 neutral, 10 unattractive images per sex) selected from the freely-available Chicago Face Database (Ma et al., 2015). All photographs included the head and shoulders of Caucasian individuals with neutral facial expressions and a constant white background. Facial stimuli were randomly presented to each participant on a computer screen, and testing was self-paced. Participants were instructed to rate each face for physical attractiveness by selecting their response on a 5-point scale presented below each image anchored at "very unattractive" (1) and "very attractive" (5). The presentation of facial stimuli was controlled by the E-Prime software package (version 2.0.1).

3.2.3.3. Drug/Non-Drug Responsiveness Task: After completing the Facial Attractiveness Test, participants were connected to physiology equipment for skin conductance level monitoring during the final behavioral task designed to assess motivational processing for both nicotine and non-nicotine rewards (see section *3.2.2. Apparatus* for details). After establishing baseline physiology, participants reviewed task instructions and were then randomly presented with a series of images from the standardized International Affective Picture System (IAPS; Lang et al., 2007) and International Smoking Image Series (ISIS; Gilbert & Rabinovich, 1998). Forty images in total were presented on the computer screen with equal numbers containing

affectively-positive, affectively-negative, neutral, or nicotine-related content. Picture cues were presented for three seconds before being replaced by a new screen asking participants to rate their emotional valence by clicking on a 1-9 scale anchored at "very unpleasant/unhappy" (1) and "very pleasant/happy" (9). After selecting their response, a new screen appeared asking participants to rate the intensity of their emotional arousal on a 1-9 scale anchored at "very unarousing/unstimulating" (1) and "very arousing/stimulating" (9). After task completion, participants were administered the Simulator Sickness Questionnaire (Kennedy et al., 1993) to address potential adverse side effects associated with completing virtual tasks and nicotine administration, including fatigue, boredom, headache, nausea, faintness, confusion, etc. Participants who indicated that they experienced any adverse symptoms were also asked to indicate symptom severity (slight, moderate, or severe).

3.3. Results

3.3.1 Participants: Of the two hundred and thirty-seven undergraduates recruited for this study, data from fourteen participants were excluded due to equipment malfunction ($n = 5$), participant compliance error (i.e. did not obey task instructions or incorrect lozenge administration; $n = 6$), or self-reported nausea ($n = 2$). There was no statistical difference between nicotine users and non-users in attrition ($n = 2$ (2.5%) in the user group and $n = 6$ (6.7%) in the non-user group; $p > 0.05$). There was also no statistical difference in attrition between those who received nicotine and those who received placebo ($n = 5$ (13.5%) in the nicotine group and $n = 1$ (3.3%) in the placebo group; $p > 0.05$). Two hundred and twenty-three participants were included in the final analysis. Of these participants, eighty-three were untreated non-users (56 female, average age = 19.1 ± 1.14), seventy-nine were untreated nicotine users (27 female, average age = 19.1 ± 1.12), twenty-nine were placebo-treated nicotine users (11 female,

average age = 19.5 ± 3.23), and thirty-two were nicotine-treated nicotine users (14 female, average age = 18.7 ± 3.21; Table 1).

	Treatment	N	Male	Female	Age (M ± SD)
Non-users	Untreated	83	27	56	19.1 ± 1.14
Users	Untreated	79	52	27	19.1 ± 1.12
	Nicotine	32	18	14	18.7 ± 3.21
	Placebo	29	18	11	19.5 ± 3.23

Table 1. *Demographic characteristics of the sample.*

No significant difference in age was observed between users and non-users ($t(160) = -0.16$; $p = 0.87$), nor between nicotine-treated and placebo-treated participants ($t(59) = 0.88$, $p = 0.38$). While participants were required to be at least six hours abstinent prior to completing the study per inclusion criteria, on average, users collapsed across treatments were 32.3 ± 52.4 hours abstinent. The number of hours since last nicotine use was not significantly different between the nicotine and placebo groups ($M_{placebo} = 36.0 \pm 32.0$, $M_{nicotine} = 46.5 \pm 34.8$; $t(59) = -0.60$, $p = 0.55$). The preferred method of nicotine consumption was e-cigarettes, which was defined as any electronic nicotine delivery system ($n = 60$). Other methods of consumption included tobacco cigarettes ($n = 23$) and chewing tobacco ($n = 5$).

3.3.2. Balloon Analogue Risk Task (BART): The effects of nicotine use status (nicotine user vs. non-user) on several BART measures were assessed using independent sample t-tests. Dependent variables included the total number of pumps, adjusted total pumps (the total number of pumps that did not result in an explosion), adjusted average pumps (the average number of pumps on balloons that did not explode), total money earned, and total number of explosions. The alpha level was adjusted to 0.01 to account for multiple tests.

Of the one hundred and nineteen untreated participants that completed the BART task for virtual rewards only, two non-users and ten users were excluded from BART analysis due to the

experimenter incorrectly entering the number of balloon trials (10) or the appropriate amount per pump (25¢). Thus, ninety-eight participants were included in the analysis ($n_{nonuser}$ = 54, n_{user} = 44). There were no significant differences between nicotine users and non-users on total number of pumps ($M_{nonuser}$ = 245.7 ± 117.4, M_{user} = 237.2 ± 108.8; $t(96) = 0.37$; $p = 0.71$), adjusted total pumps ($M_{nonuser}$ = 183.2 ± 73.2, M_{user} = 174.6 ± 70.3; $t(96) = 0.59$; $p = 0.56$), adjusted average pumps ($M_{nonuser}$ = 24.8 ± 11.8, M_{user} = 23.8 ± 10.9; $t(96) = 0.40$; $p = 0.68$), total money earned ($M_{nonuser}$ = 4581.0 ± 1829.5, M_{user} = 4365.0 ± 1757.3; $t(96) = 0.59$; $p = 0.56$), and total number of explosions ($M_{nonuser}$ = 2.07 ± 1.40, M_{user} = 2.14 ± 1.42; $t(96) = -0.22$; $p = 0.83$; Table 2; Figure 3a).

BART Measure	Non-user (M ± SD)	User (M ± SD)	*t*	*df*	*p*
Total pumps	245.7 ± 117.4	237.2 ± 108.8	0.37	96	0.71
Adjusted total	183.2 ± 73.2	174.6 ± 70.3	0.59	96	0.56
Adjusted average	24.8 ± 11.8	23.8 ± 10.9	0.40	96	0.68
Total money earned	4581.0 ± 1829.5	4365.0 ± 1757.3	0.59	96	0.56
Total explosions	2.07 ± 1.40	2.14 ± 1.42	-0.22	96	0.83

Table 2. *No significant differences between nicotine users and non-users on the total number of pumps, adjusted total number of pumps, adjusted average pumps, total money earned, and total number of explosions in the virtual reward version of the BART task.*

Of the fifty-two participants that completed the BART task with the potential to earn a $20 Amazon gift card, one non-user was excluded from BART analysis due to the experimenter incorrectly entering the appropriate amount per pump. Thus, fifty-one participants were included in the analysis ($n_{nonuser}$ = 26, n_{user} = 25). Again, there were no significant differences between nicotine users and non-users on total number of pumps ($M_{nonuser}$ = 263.4 ± 161.2, M_{user} = 250.3 ± 135.8; $t(49) = 0.31$; $p = 0.17$), adjusted total pumps ($M_{nonuser}$ = 170.4 ± 85.6, M_{user} = 182.0 ± 68.4; $t(49) = -0.54$; $p = 0.59$), adjusted average pumps ($M_{nonuser}$ = 27.4 ± 18.7, M_{user} = 26.0 ± 15.3; $t(49) = 0.28$; $p = 0.78$), total money earned ($M_{nonuser}$ = 4258.6 ± 2141.0, M_{user} = 4551.0. ± 1710.6;

$t(49) = -0.54$; $p = 0.59$), and total number of explosions ($M_{nonuser} = 2.69 \pm 1.98$, $M_{user} = 2.16 \pm 1.70$; $t(49) = 1.03$; $p = 0.30$; Table 3; Figure 3b).

BART Measure	Non-user (M ± SD)	User (M ± SD)	*t*	*df*	*p*
Total pumps	263.4 ± 161.2	250.3 ± 135.8	0.31	49	0.17
Adjusted total	170.3 ± 85.6	182.0 ± 68.4	-0.54	49	0.59
Adjusted average	27.4 ± 18.7	26.0 ± 15.3	0.28	49	0.78
Total money earned	4258.6 ± 2141.0	4551.0 ± 1710.6	-0.54	49	0.59
Total explosions	2.69 ± 1.98	2.16 ± 1.70	1.03	49	0.30

Table 3. *There were no significant differences between nicotine users and non-users on the total number of pumps, adjusted total number of pumps, adjusted average pumps, total money earned, and total number of explosions in the version of the BART task with the potential to earn a real monetary gift card.*

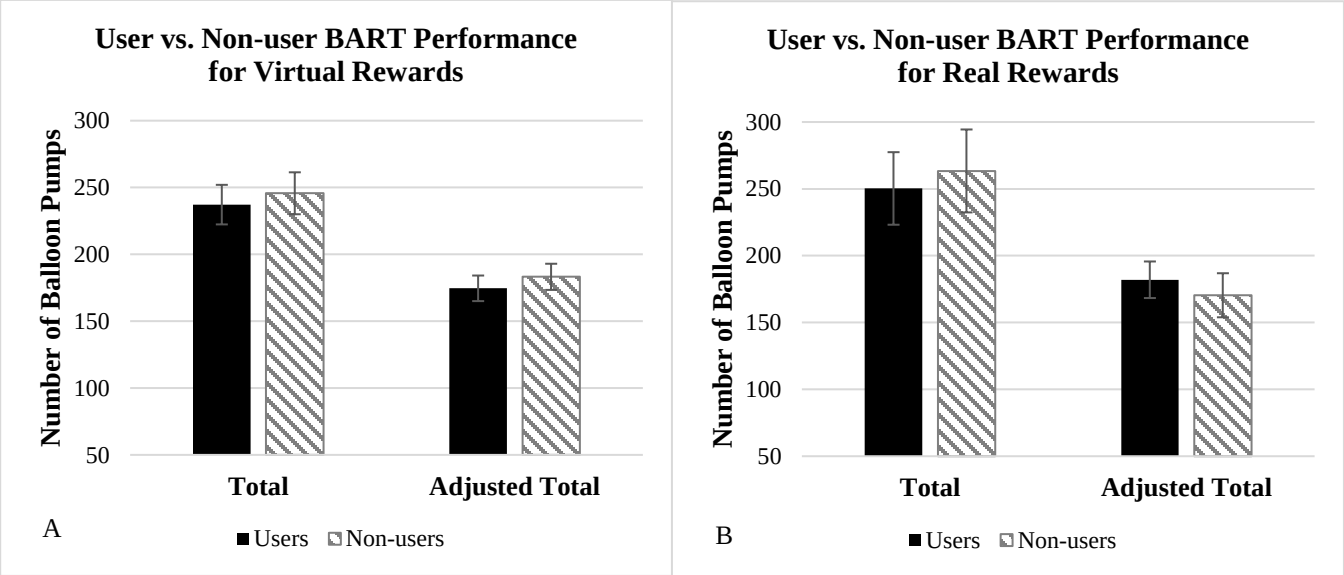


Figure 3a. *No significant difference between users and non-users on balloon pumps ($t(96) = 0.37$; $p = 0.71$) or adjusted total balloon pumps ($t(96) = 0.59$; $p = 0.56$) for virtual rewards.*

Figure 3b. *No significant difference between users and non-users on total balloon pumps ($t(49) = 0.31$; $p = 0.76$) or adjusted total balloon pumps ($t(49) = -0.54$; $p = 0.59$) for real monetary rewards.*

One-way ANOVAs were then conducted between the two BART experiments to determine whether the potential for real earnings differentially affected the outcome variables relative to

virtual earnings. Among non-users, there was no significant effect of reward type on total number of pumps ($M_{virtual}$ = 245.7 ± 117.4, M_{real} = 263.4 ± 161.2; $F(1, 79) = 0.31$, $p = 0.58$), adjusted total pumps ($M_{virtual}$ = 183.2 ± 73.2, M_{real} = 170.4 ± 85.6; $F(1, 79) = 0.49$, $p = 0.49$), adjusted average pumps ($M_{virtual}$ = 24.8 ± 11.8, M_{real} = 27.4 ± 18.7; $F(1, 79) = 0.59$, $p = 0.45$), total money earned ($M_{virtual}$ = 4581.0 ± 1829.5, M_{real} = 4258.7 ± 2141.0; $F(1, 79) = 0.49$, $p = 0.49$), and total number of explosions ($M_{virtual}$ = 2.07 ± 1.40, M_{real} = 2.69 ± 1.98; $F(1, 79) = 2.60$, $p = 0.11$; Table 4).

BART Measure	Virtual (M ± SD)	Real (M ± SD)	*F*	*df*	*p*
Total pumps	245.7 ± 117.4	263.4 ± 161.2	0.31	1, 79	0.58
Adjusted total	183.2 ± 73.2	170.4 ± 85.6	0.49	1, 79	0.49
Adjusted average	24.8 ± 11.8	27.4 ± 18.7	0.59	1, 79	0.45
Total money earned	4581.0 ± 1829.5	4258.7 ± 2141.0	0.49	1, 79	0.49
Total explosions	2.07 ± 1.40	2.69 ± 1.98	2.60	1, 79	0.11

Table 4. *Among non-users, no differences were found between the two versions of the BART experiment regardless of whether participants were working for the potential to receive real monetary earnings or for virtual earnings.*

Likewise among nicotine users, there was no significant effect of reward type on total number of pumps ($M_{virtual}$ = 237.2 ± 108.8, M_{real} = 250.3 ± 135.7; $F(1, 68) = 0.19$, $p = 0.66$), adjusted total pumps ($M_{virtual}$ = 174.6 ± 70.3, M_{real} = 182.0 ± 68.4; $F(1, 68) = 0.18$, $p = 0.67$), adjusted average pumps ($M_{virtual}$ = 23.7 ± 12.2, M_{real} = 26.0 ± 15.3; $F(1, 68) = 0.44$, $p = 0.49$), total money earned ($M_{virtual}$ = 4365.3 ± 1757.3, M_{real} = 4551.0 ± 1710.6; $F(1, 68) = 0.18$, $p = 0.67$), and total number of explosions ($M_{virtual}$ = 2.14 ± 1.42, M_{real} = 2.16 ± 1.70; $F(1, 68) = 0.004$, $p = 0.95$; Table 5).

BART Measure	Virtual (M ± SD)	Real (M ± SD)	*F*	*df*	*p*
Total pumps	237.2 ± 108.8	250.3 ± 135.7	0.19	1, 68	0.66
Adjusted total	174.6 ± 70.3	182.0 ± 68.4	0.18	1, 68	0.67
Adjusted average	23.7 ± 12.2	26.0 ± 15.3	0.44	1, 68	0.49

Total money earned	4365.3 ± 1757.3	4551.0 ± 1710.6	0.18	1, 68	0.67
Total explosions	2.14 ± 1.42	2.16 ± 1.70	0.004	1, 68	0.95

Table 5. *Among users, no differences were found between the two versions of the BART experiment regardless of whether participants were working for the potential to receive real monetary earnings or for virtual earnings.*

Data collection was only conducted using virtual rewards to assess differences in risk-taking between placebo-treated and nicotine-treated users. Of the sixty-one participants that completed the task, three placebo-treated participants were excluded due to the experimenter incorrectly entering the number of balloon trials (10) or the appropriate amount per pump (25¢). Thus, fifty-seven participants were included in the analysis ($n_{placebo}$ = 26, $n_{nicotine}$ = 32). Independent sample t-tests revealed that relative to placebo, nicotine administration had no significant effect on total number of pumps ($M_{placebo}$ = 268.1 ± 116.4, $M_{nicotine}$ = 311.8 ± 212.5; $t(56) = -0.94$, $p = 0.35$), adjusted total pumps ($M_{placebo}$ = 189.9 ± 60.4, $M_{nicotine}$ = 225.63 ± 144.5; $t(56) = -1.19$, $p = 0.24$), adjusted average ($M_{placebo}$ = 27.8 ± 13.0, $M_{nicotine}$ = 28.7 ± 11.8; $t(56) = -0.27$, $p = 0.79$), total money earned ($M_{placebo}$ = 4739.4 ± 1509.0, $M_{nicotine}$ = 5640.6 ± 3611.9; $t(56) = -1.19$, $p = 0.24$), and total number of explosions ($M_{placebo}$ = 2.65 ± 1.34, $M_{nicotine}$ = 2.66 ± 1.81; $t(56) = -0.006$, $p = 0.99$; Table 6; Figure 4).

BART Measure	Placebo (M ± SD)	Nicotine (M ± SD)	*t*	*df*	*p*
Total pumps	268.1 ± 116.4	311.8 ± 212.5	-0.94	56	0.35
Adjusted total	189.9 ± 60.4	225.63 ± 144.5	-1.19	56	0.24
Adjusted average	27.8 ± 13.0	28.7 ± 11.8	-0.27	56	0.79
Total money earned	4739.4 ± 1509.0	5640.6 ± 3611.9	-1.19	56	0.24
Total explosions	2.65 ± 1.34	2.66 ± 1.81	-0.006	56	0.99

Table 6. *No significant differences between nicotine- and placebo-treated users on the total number of pumps, adjusted total number of pumps, adjusted average pumps, total money earned, and total number of explosions in the virtual reward version of the BART task.*

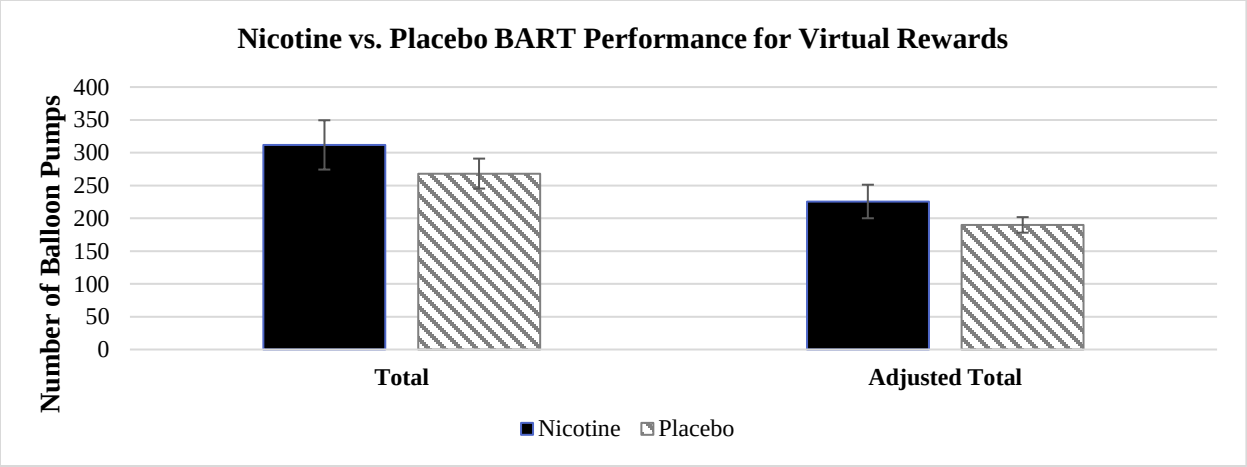


Figure 4. *No significant difference between nicotine and placebo groups on total balloon pumps (*t(56) = -0.94, p = 0.35*) or adjusted total balloon pumps (*t(56) = -1.19, p = 0.24*) for virtual rewards.*

3.3.3. Facial Attractiveness Test: Six nicotine users were excluded from Facial Attractiveness Test analysis due to equipment malfunction or experimenter error. Thus, one hundred and fifty-six participants were included in the final analysis ($n_{nonuser}$ = 79, n_{user} = 77). The effects of nicotine use status on attractiveness ratings of all facial stimuli, same sex stimuli (where participant sex and image sex were the same), and opposite sex stimuli (where participant sex and image sex were opposite) were analyzed using independent sample t-tests. The alpha level was adjusted to 0.01 to account for multiple tests. Analyses revealed no significant effect of nicotine use status on ratings of same sex images ($M_{nonuser}$ = 2.31 ± 0.56, M_{user} = 2.18 ± 0.64; t(155) = 1.33; p = 0.19), opposite sex images ($M_{nonuser}$ = 2.14 ± 0.48, M_{user} = 2.23 ± 0.54; t(155) = -1.16; p = 0.25), or all images when sex was collapsed ($M_{nonuser}$ = 2.22 ± 0.49, M_{user} = 2.23 ± 0.47; t(155) = -0.16; p = 0.88; Table 7).

Facial Attractiveness Measure	Non-user (M ± SD)	User (M ± SD)	*t*	*df*	*p*
Same sex	2.31 ± 0.56	2.18 ± 0.64	1.33	155	0.19
Opposite sex	2.14 ± 0.48	2.23 ± 0.54	-1.16	155	0.25
All images	2.22 ± 0.49	2.23 ± 0.47	-0.16	155	0.88

Table 7. *No significant effect of nicotine use status on absolute ratings of same sex images (t(155) = 1.33; p = 0.19), opposite sex images (t(155) = -1.16; p = 0.25), or all images when sex was collapsed (t(155) = -0.16; p = 0.88).*

Absolute scores of facial attractiveness were then transformed to difference scores by subtracting the participant's rating from the standardized rating, such that positive difference scores indicated more favorable ratings by the participant than standard. Independent sample t-tests again revealed no effect of nicotine use status on ratings difference scores of same sex ($M_{nonuser}$ = -0.94 ± 0.52, M_{user} = -0.92 ± 0.56; $t(155)$ = -0.14; p = 0.89), opposite sex ($M_{nonuser}$ = -1.00 ± 0.49, M_{user} = -0.97 ± 0.45; $t(155)$ = -0.47; p = 0.64), or all images collapsed across sex ($M_{nonuser}$ = -0.97 ± 0.48, M_{user} = -0.96 ± 0.47; $t(155)$ = -0.15; p = 0.88; Table 8).

Facial Attractiveness Measure	Non-user (M ± SD)	User (M ± SD)	*t*	*df*	*p*
Same sex	-0.94 ± 0.52	-0.92 ± 0.56	-0.14	155	0.89
Opposite sex	-1.00 ± 0.49	-0.97 ± 0.45	-0.47	155	0.64
All images	-0.97 ± 0.48	-0.96 ± 0.47	-0.15	155	0.88

Table 8. *No significant effect of nicotine use status on ratings difference scores of same sex images (t(155) = -0.14; p = 0.89), opposite sex images (t(155) = -0.47; p = 0.64), or all images when sex was collapsed (t(155) = -0.15; p = 0.88).*

The same analyses were repeated to evaluate the effect of acute nicotine administration on facial attractiveness ratings relative to placebo. One placebo-treated participant was excluded from analysis due to experimenter error. Therefore, sixty participants were included in the analysis ($n_{placebo}$ = 28, $n_{nicotine}$ = 32). Analyses revealed no significant effect of nicotine administration on absolute ratings of same sex ($M_{placebo}$ = 2.20 ± 0.56, $M_{nicotine}$ = 2.07 ± 0.61; $t(58)$ = 0.84; p = 0.40), opposite sex ($M_{placebo}$ = 2.19 ± 0.53, $M_{nicotine}$ = 2.10 ± 0.53; $t(58)$ = 0.63; p

= 0.53), or all images when sex was collapsed ($M_{placebo}$ = 2.19 ± 0.50, $M_{nicotine}$ = 2.09 ± 0.40; $t(58) = 0.84$; $p = 0.40$; Table 9).

Facial Attractiveness Measure	Placebo (M ± SD)	Nicotine (M ± SD)	*t*	*df*	*p*
Same sex	2.20 ± 0.56	2.07 ± 0.61	0.84	58	0.40
Opposite sex	2.19 ± 0.53	2.10 ± 0.53	0.63	58	0.53
All images	2.19 ± 0.50	2.09 ± 0.40	0.84	58	0.48

Table 9. *No significant effect of treatment on absolute ratings of same sex images ($t(58) = 0.84$; $p = 0.48$), opposite sex images ($t(58) = 0.63$; $p = 0.53$), or all images when sex was collapsed ($t(58) = 0.84$; $p = 0.48$).*

Additionally, no differences between treatments were observed when using ratings difference scores of same sex ($M_{placebo}$ = -0.90 ± 0.58, $M_{nicotine}$ = -1.10 ± 0.60; $t(58) = 0.92$; p = 0.36), opposite sex ($M_{placebo}$ = -1.03 ± 0.47, $M_{nicotine}$ = -1.11 ± 0.46; $t(58) = 0.60$; $p = 0.55$), or all images ($M_{placebo}$ = -1.00 ± 0.50, $M_{nicotine}$ = -1.10 ± 0.49; $t(58) = 0.83$; $p = 0.41$; Table 10). .

Facial Attractiveness Measure	Placebo (M ± SD)	Nicotine (M ± SD)	*t*	*df*	*p*
Same sex	-0.90 ± 0.58	-1.10 ± 0.60	0.92	58	0.36
Opposite sex	-1.03 ± 0.47	-1.11 ± 0.46	0.60	58	0.55
All images	-1.00 ± 0.50	-1.10 ± 0.49	0.83	58	0.41

Table 10. *No significant effect of treatment on ratings difference scores of same sex images ($t(58) = 0.92$; $p = 0.36$), opposite sex images ($t(58) = 0.60$; $p = 0.55$), or all images when sex was collapsed ($t(58) = 0.83$; $p = 0.41$).*

3.3.4. Drug/Non-Drug Responsiveness Task:

Nicotine users vs. Non-users: Four non-users and one nicotine user were excluded from task analysis due to equipment malfunction or experimenter error. One hundred and fifty-seven participants were included in the analysis ($n_{nonuser}$ =79, n_{user} = 78). To evaluate responses to nicotine-related cues and non-nicotine affective stimuli, participants subjectively rated each stimulus twice, once in terms of emotional valence (pleasurable vs. unpleasurable) and again in terms of arousal (intensity of the emotional response). Independent sample t-tests were conducted to assess differences between nicotine users and non-users on these subjective ratings

measures. Images were categorized as nicotine-related, affectively-positive, affectively-negative, and neutral. To account for multiple comparisons, alpha was adjusted to 0.01. Means and standard deviations are summarized in Table 11.

Relative to non-users, nicotine users rated nicotine-associated images as significantly more pleasurable ($t(155) = -5.42$, $p = 0.0001$; Figure 5a) with a trend to also rate them more arousing ($t(155) = -1.88$, $p = 0.06$; Figure 5b). There was no effect of nicotine use status on valence ratings of positive ($t(155) = -1.16$, $p = 0.11$), negative ($t(155) = -0.28$, $p = 0.78$), nor neutral images ($t(155) = 0.92$, $p = 0.36$; Figure 5a). Similarly, no differences were observed on arousal ratings of positive ($t(155) = -0.43$, $p = 0.67$), negative ($t(155) = 0.47$, $p = 0.63$), nor neutral cues ($t(155) = -0.12$, $p = 0.91$; Figure 5b).

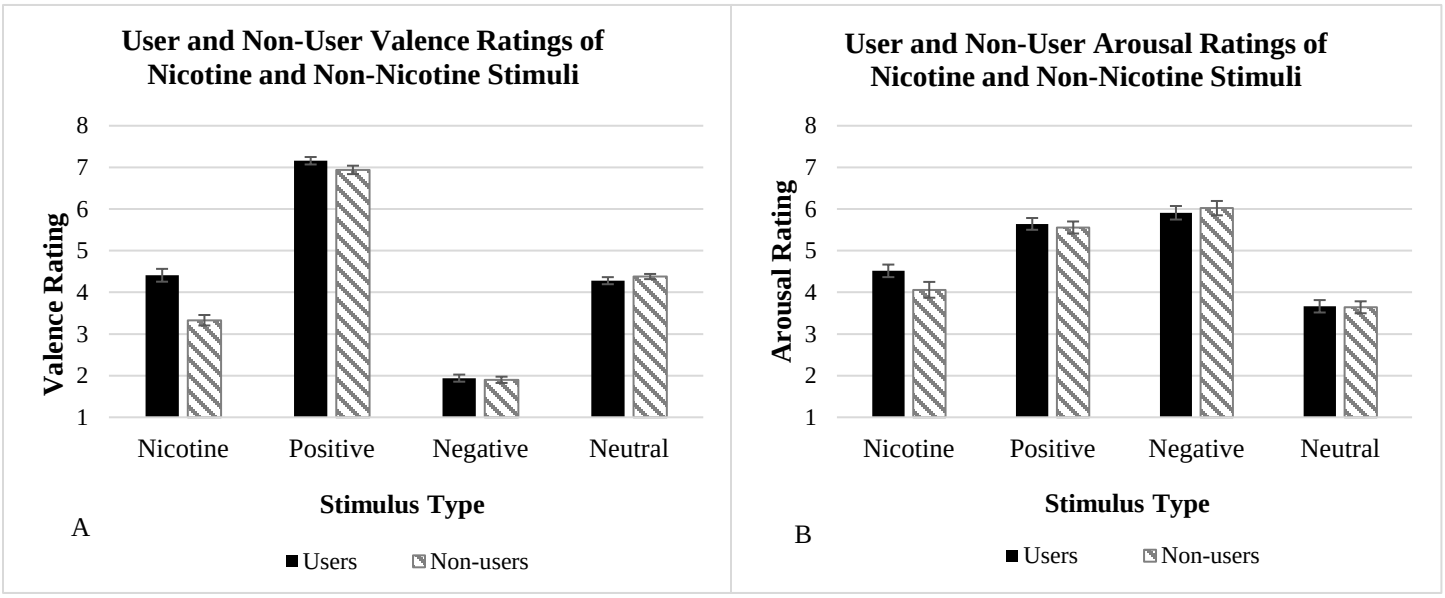


Figure 5. *Drug/Non-Drug Responsiveness Task performance showing (a) valence ratings and (b) arousal ratings for nicotine-related, positive, negative, and neutral stimuli among nicotine users and non-users.*

Physiological electrodermal data were visually inspected and corrected for artifacts before statistical analysis. Specifically, data were resampled to 62.5 samples/second and median value baseline smoothing was applied to eliminate rapid transient events. To correct for high frequency

electrical noise and movement artifacts, a low pass filter optimized for the sample rate cutoff was applied. The change in skin conductance response (SCR) was calculated for each image by subtracting the mean baseline skin conductance level during the 1s immediately preceding stimulus presentation from the maximum skin conductance level recorded 2-5s following stimulus onset. This recording interval was chosen to avoid confounding recordings with the presentation of the following image. Image cues were presented for 3s before being replaced by a new screen asking participants to make their valence rating. After making their rating, participants were asked to score the emotional intensity of their previous rating. Therefore, ratings were self-paced and intertrial intervals varied. Manual scoring of SCRs ensured no overlap with neighboring responses and confirmed that 2-5s was an appropriate window. Others have indicated that the initial inflection point of a conductance response occurs within 1-4s following cue onset, and that the mean peak latency ranges from 2-6s (Pineles et al., 2009). Moreover, this scoring method has been validated across numerous human physiological publications (Lonsdorf et al., 2017; Boucsein, 2012; Dawson et al., 2007).

Independent sample t-tests were performed to explore SCR differences between nicotine users and non-users to each stimulus type. Physiological data from twenty-six non-users and twenty nicotine users were excluded from analysis due to equipment malfunction, poor electrode adhesion, or participant withdrawal from the study. Data from one hundred and sixteen participants were included in the analysis ($n_{\text{nonuser}} = 57$, $n_{\text{user}} = 59$). Compared to non-users, users exhibited significantly greater SCRs when rating nicotine-related images ($t(114) = -3.07$; $p = 0.003$; Figure 6). No significant SCR differences were observed between users and non-users

when rating positive ($t(114) = 1.36$; $p = 0.18$), negative ($t(114) = -0.62$; $p = 0.54$), nor neutral images ($t(114) = 1.26$; $p = 0.21$; Figure 6).

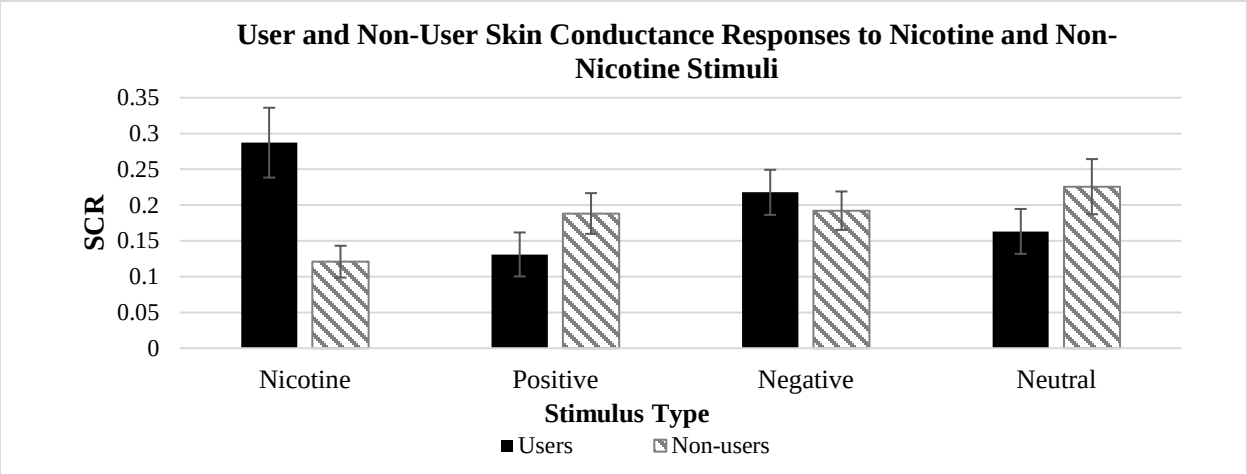


Figure 6. *Drug/Non-Drug Responsiveness Task performance showing skin conductance responses to nicotine-related, positive, negative, and neutral stimuli among nicotine users and non-users.*

To address our *a priori* hypothesis that nicotine users would assign enhanced motivational salience to nicotine-paired cues relative to affectively-negative and neutral cues, paired sample t-tests were conducted between SCRs and subjective ratings of these image types, respectively. Within untreated nicotine users, nicotine-related stimuli were rated as significantly more pleasurable than negative affective images ($t(77) = -15.3$; $p = 0.0001$; Figure 5a). However, arousal data indicated that users rated negative images more arousing than nicotine images ($t(77) = 8.39$; $p = 0.0001$), while no differences in SCRs were observed between the image types ($t(59) = -1.52$; $p = 0.13$; Figure 6). Additionally, there were no differences in user valence ratings between nicotine and neutral images ($t(77) = -0.92$; $p = 0.36$; Figure 5a); however nicotine images were rated as significantly more arousing than neutral images ($t(77) = -6.57$; $p = 0.0001$; Figure 5b) and SCRs were significantly greater for nicotine images than neutral images ($t(59) = -2.51$; $p = 0.001$; Figure 6). Negative images were also rated as more arousing than neutral images ($t(77) = 13.22$; $p = 0.0001$; Figure 5b); however, valence ratings were higher for negative images

than neutral images ($t(77) = -26.5$; $p = 0.0001$; Figure 5a).

Exploring the direction of incentive salience attribution between nicotine and non-nicotine rewards among users, we observed that positive images were rated as significantly more pleasurable ($t(77) = 16.7$; $p = 0.0001$; Figure 5a) and more arousing than nicotine images ($t(77) = 8.08$; $p = 0.0001$; Figure 5b). Conversely, SCRs were significantly greater to nicotine images compared to positive images ($t(58) = -3.51$; $p = 0.001$; Figure 6). While positive images were rated as significantly more pleasurable than negative images ($t(77) = 36.65$; $p = 0.0001$, Figure 5a), there was a trend for SCRs to be significantly greater for negative images than positive images ($t(58) = -2.278$; $p = 0.03$; Figure 5b). Nicotine users exhibited no difference in arousal ($t(77) = -1.70$; $p = 0.09$; Figure 5b) between positive and negative images. Positive images were also rated significant more pleasant ($t(77) = 36.7$; $p = 0.0001$; Figure 5a) and arousing ($t(77) = 15.1$; $p = 0.0001$; Figure 5b) than neutral images. However, there were no differences in SCRs between positive and neutral images among users ($t(58) = -0.96$; $p = 0.34$; Figure 6).

Within non-users, the valence of positive images was rated significantly higher than negative ($t(78) = 32.96$; $p = 0.0001$), neutral ($t(78) = 23.36$; $p = 0.0001$), and nicotine images ($t(78) = 20.79$; $p = 0.0001$; Figure 5a). The arousal of positive images was also rated significantly higher than neutral ($t(78) = 15.89$; $p = 0.0001$) and nicotine images ($t(78) = 8.4$; $p = 0.0001$); however, negative images were rated more arousing than positive images among non-users ($t(78) = -2.45$; $p = 0.02$; Figure 5b). The valence of neutral images was rated significantly higher than both negative ($t(78) = -30.14$; $p = 0.0001$) and nicotine images ($t(78) = 8.78$; $p = 0.0001$); however, nicotine images were rated as more pleasurable than negative images ($t(78) = -13.09$; $p = 0.0001$; Figure 5a). In terms of arousal, negative images had higher non-user arousal ratings than neutral ($t(78) = 12.76$; $p = 0.0001$) and nicotine images ($t(78) = 12.68$; $p = 0.0001$), while nicotine

images had higher arousal ratings than neutral images ($t(78) = -2.68$; $p = 0.01$; Figure 5b).

In terms of physiological responses, neutral images produced significantly greater SCRs than nicotine images in non-users ($t(56) = 2.66$; $p = 0.01$; Figure 6). There was also a non-significant trend for non-users to display higher SCRs to positive ($t(56) = 2.30$; $p = 0.03$) and negative stimuli ($t(56) = 2.29$; $p = 0.03$) relative to nicotine cues. There were no significant differences in SCRs between positive and negative ($t(56) = -0.14$; $p = 0.89$), positive and neutral images ($t(56) = -0.91$; $p = 0.37$), nor negative and neutral images ($t(56) = -0.81$; $p = 0.42$; Figure 6).

	Valence Ratings		***Arousal Ratings***		***SCR***	
	Users	**Non-users**	**Users**	**Non-users**	**Users**	**Non-users**
Positive	7.16 ± 0.79	6.93 ± 0.90	5.64 ± 1.25	5.55 ± 1.28	0.13 ± 0.24	0.19 ± 0.21
Negative	1.94 ± 0.75	1.90 ± 0.67	5.91 ± 1.43	6.02 ± 1.53	0.22 ± 0.24	0.19 ± 0.20
Neutral	4.28 ± 0.75	4.38 ± 0.55	3.67 ± 1.31	3.64 ± 1.28	0.16 ± 0.24	0.23 ± 0.29
Nicotine	4.41 ± 1.36	3.33 ± 1.12	4.52 ± 1.33	4.06 ± 1.69	0.29 ± 0.37	0.12 ± 0.17

Table 11. *Group means and standard deviations for valence ratings, arousal rating, and skin conductance responses of all stimulus types for nicotine users and non-users.*

Nicotine vs. Placebo: To assess the effects of acute nicotine administration on reward responsiveness relative to placebo, independent sample t-tests were conducted between treatments using valence and arousal ratings, as well as physiological measures. Three placebo-treated participants and one nicotine-treated participant were excluded from analysis due to equipment malfunction or experimenter error resulting in fifty-seven participants included in the final analysis ($n_{placebo} = 26$, $n_{nicotine} = 31$). Means and standard deviations are summarized in Table 12.

While we theorized that nicotine administration relative to placebo would magnify the hypothesized outcomes between nicotine users and non-users, no significant differences were observed between treatment groups on any metrics for any image type: valence ratings (Figure 7a), arousal ratings (Figure 7b), and physiological measures (Figure 8). Within subjects analyses

for the nicotine and placebo groups, respectively, were conducted to compare the magnitude of incentive salience assigned to nicotine cues relative to affective and neutral non-drug stimuli. Interestingly, both nicotine- and placebo-treated participants exhibited the same pattern of valence ratings as untreated nicotine users from the previous experiment sample. Specifically, the nicotine group rated positive images as significantly more pleasurable than negative ($t(30) = 21.9$, $p = 0.0001$), neutral ($t(30) = 18.5$, $p = 0.0001$), and nicotine images ($t(30) = 12.6$, $p = 0.0001$; Figure 8a). Both nicotine ($t(30) = -10.8$, $p = 0.0001$) and neutral images ($t(30) = -12.9$, $p = 0.0001$) were rated higher than negative images, but no difference was found between neutral and nicotine cues ($t(30) = -1.59$, $p = 0.12$; Figure 7a).

Placebo-treated participants also rated positive images as significantly more pleasurable than negative ($t(25) = 20.6$, $p = 0.0001$), neutral ($t(30) = 13.3$, $p = 0.0001$), and nicotine images ($t(25) = 9.95$, $p = 0.0001$; Figure 7a). Neutral images ($t(25) = -14.2$, $p = 0.0001$) and nicotine images ($t(25) = -10.0$, $p = 0.0001$) were rated higher than negative images, but no difference was found between neutral and nicotine cues ($t(25) = -0.89$, $p = 0.38$; Figure 7a).

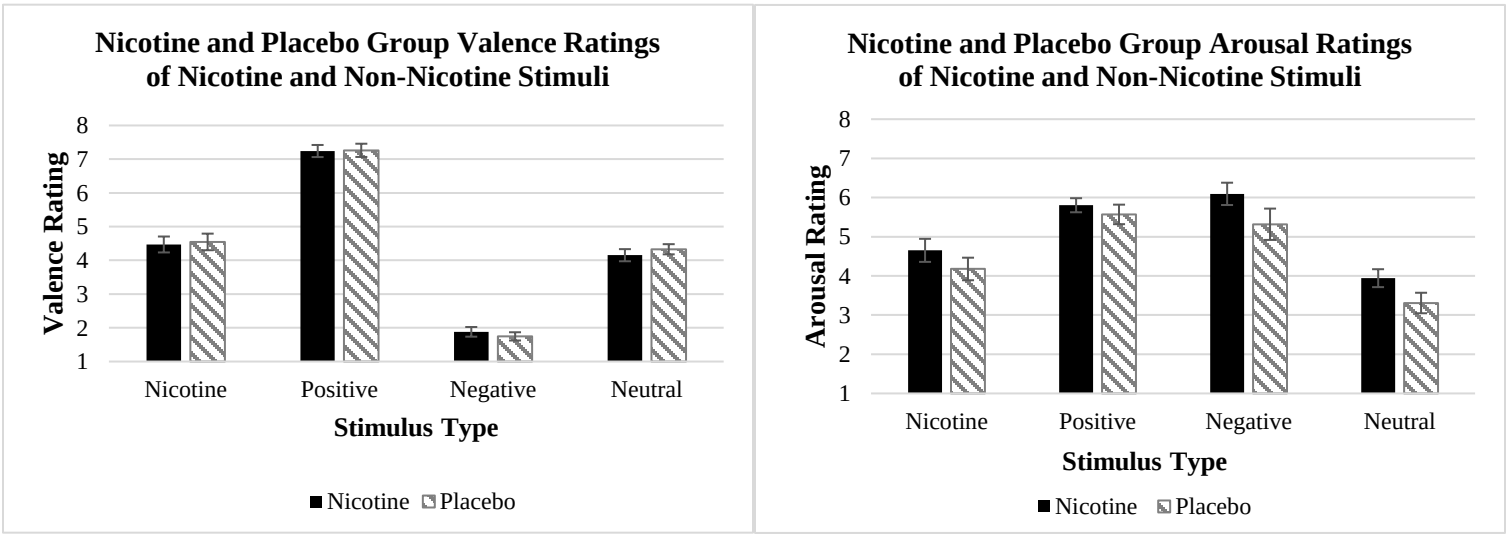


Figure 7. *Drug/Non-Drug Responsiveness Task performance showing (a) valence ratings and (b) arousal ratings for nicotine-related, positive, negative, and neutral stimuli among nicotine-treated and placebo-treated participants.*

In terms of arousal, nicotine-treated participants rated positive images as significantly more arousing than neutral $t(30) = 8.99$, $p = 0.0001$) and nicotine stimuli ($t(30) = 1.57$, $p = 0.0001$; Figure 7b). Negative images were also rated as more arousing than neutral ($t(30) = 8.01$, $p = 0.0001$) and nicotine stimuli ($t(25) = 4.54$, $p = 0.0001$); however, nicotine cues were more stimulating than neutral cues among the nicotine group ($t(30) = -3.19$, $p = 0.003$; Figure 7b).

Placebo-treated users expressed the same pattern of arousal results. Positive images were significantly more arousing than neutral ($t(25) = 8.45$, $p = 0.0001$) and nicotine stimuli ($t(25) = 4.71$, $p = 0.0001$; Figure 7b). Negative images were also rated more arousing than neutral ($t(25) = 5.95$, $p = 0.0001$) and nicotine stimuli ($t(25) = 1.85$, $p = 0.003$); however, nicotine cues were more stimulating than neutral cues among the placebo group ($t(25) = -3.07$, $p = 0.005$; Figure 7b). Finally, there were no differences in SCRs between image types when analyzing within the nicotine group and the placebo group, respectively (Figure 8).

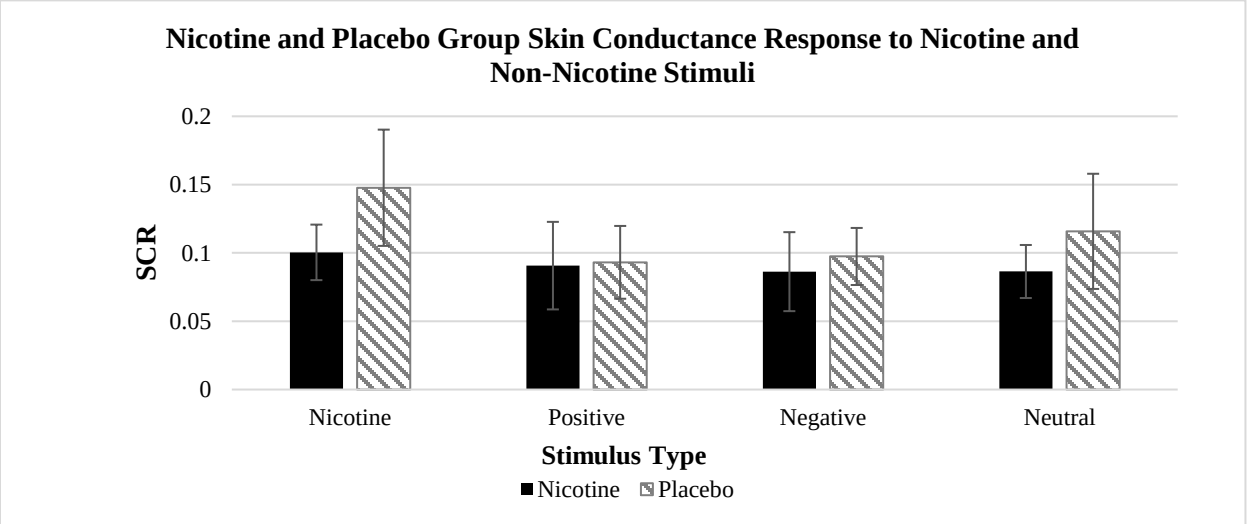


Figure 8. *Drug/Non-Drug Responsiveness Task performance showing skin conductance responses to nicotine-related, positive, negative, and neutral stimuli among nicotine-treated and placebo-treated participants.*

	Valence Ratings		*Arousal Ratings*		*SCR*	
	Nicotine	**Placebo**	**Nicotine**	**Placebo**	**Nicotine**	**Placebo**
Positive	7.24 ± 1.00	7.26 ± 1.00	5.80 ± 1.00	5.57 ± 1.27	0.09 ± 0.17	0.09 ± 0.13
Negative	1.88 ± 0.80	1.75 ± 0.62	6.09 ± 1.58	5.32 ± 2.04	0.09 ± 0.25	0.09 ± 0.10
Neutral	4.15 ± 1.00	4.32 ± 0.77	3.94 ± 1.27	3.31 ± 1.33	0.09 ± 0.10	0.12 ± 0.21
Nicotine	4.47 ± 1.31	4.54 ± 1.16	4.65 ± 1.64	4.18 ± 1.47	0.10 ± 0.11	0.15 ± 0.21

Table 12. *Group means and standard deviations for valence ratings, arousal rating, and skin conductance responses of all stimulus types for nicotine-treated and placebo-treated participants.*

Finally, to investigate the relationship between ratings and physiological data, significant positive correlations were found between valence and arousal ratings of positive ($r(214) = 0.56$; $p = 0.0001$) and nicotine stimuli ($r(214) = 0.18$; $p = 0.009$), while a significant negative relationship was found between valence and arousal of negative images ($r(214) = -0.23$; $p = 0.0001$). SCRs did not correlate with valence or arousal for any image type.

3.3.5. Questionnaire Results: Independent sample t-tests were conducted to compare differences on questionnaire metrics between nicotine users and non-users, and between nicotine-treated and placebo-treated participants. The alpha level was adjusted to 0.01 to account for multiple tests. Logically, nicotine users were significantly more nicotine dependent than non-users as measured by the Fagerstrom Test for Nicotine Dependence (FTND; $M_{nonuser} = 0.05 \pm 0.44$, $M_{user} = 1.65 \pm 2.06$; $t(160) = -6.89$; $p = 0.0001$). There was no difference between the nicotine group and placebo group on Fagerstrom score ($M_{placebo} = 1.66 \pm 1.88$, $M_{nicotine} = 1.94 \pm 2.31$; $t(59) = -0.52$, $p = 0.61$). Given the adjusted alpha, there was only a trend for higher scores on the Minnesota Nicotine Withdrawal scale among users relative to nonusers (MNWS; $M_{nonuser} = 13.3 \pm 8.67$, $M_{user} = 19.9 \pm 10.9$; $t(50) = -2.43$; $p = 0.02$). No differences in Withdrawal score were reported between the nicotine and placebo groups ($M_{placebo} = 20.9 \pm 9.51$, $M_{nicotine} = 18.8 \pm 10.6$; $t(59) = -0.80$, $p = 0.42$). There were no differences observed between users and non-users

on the Negative affect subscale ($M_{nonuser}$ = 6.89 ± 7.46, M_{user} = 7.62 ± 8.72; $t(160) = -0.57$; p = 0.57) nor Positive affect subscale ($M_{nonuser}$ = 9.71 ± 10.4, M_{user} = 11.0 ± 12.6; $t(160) = -0.73$; p = 0.46) of the Positive and Negative Affective schedule (PANAS). Similarly, no differences in Negative ($M_{placebo}$ = 20.2 ± 7.08, $M_{nicotine}$ = 17.0 ± 4.76; $t(59) = 2.11$, p = 0.04) nor Positive ($M_{placebo}$ = 27.6 ± 7.86, $M_{nicotine}$ = 27.7 ± 9.61; $t(59) = -0.03$, p = 0.98) affective responses were found for the nicotine and placebo groups. Positive affect corresponds with the extent to which a person feels enthusiastic, active, and alert, while negative affect is associated with feelings of distress and displeasure (Watson & Clark et al., 1988). Finally, there was no significant difference in post-test nausea measures between nicotine- and placebo-treated participants ($M_{placebo}$ = 23.1 ± 34.9, $M_{nicotine}$ = 20.4 ± 30.9; $t(59) = 0.33$, p = 0.74). This metric was not administered during the experiment phase comparing users and non-users.

Examining measures of impulsive personality characteristics, nicotine users scored significantly higher than non-users on the Disinhibition ($M_{nonuser}$ = 4.84, M_{user} = 6.14; $t(160) = -3.88$; p = 0.0001) and Experience Seeking ($M_{nonuser}$ = 4.22, M_{user} = 5.48; $t(160) = -4.66$; p = 0.0001) subscales of the Sensation Seeking Scale (SSS) questionnaire, and had significantly greater total scores ($M_{nonuser}$ = 17.5, M_{user} = 21.3; $t(160) = -4.53$; p = 0.0001). The Disinhibition subscale is associated with greater risk of participating in drug use, alcohol use, vandalism, and/or unsafe sex, while the Experience Seeking subscale relates to the pursuit of an unconventional lifestyle via unplanned activities and/or hallucinatory drugs (Zuckerman, 2007). Nicotine users also scored higher than non-users on the Motor ($M_{nonuser}$ = 21.2, M_{user} = 22.5; $t(160) = -2.41$; p = 0.01) and Non-Planning ($M_{nonuser}$ = 23.6, M_{user} = 25.3; $t(160) = -2.71$; p = 0.01) subscales of the Barratt Impulsiveness Scale. The Motor impulsiveness subscale assesses the tendency to act without thinking, while the Non-Planning subscale is attributed to lack of

future planning and forethought. There were no differences between nicotine- and placebo-treated participants on any impulsivity questionnaire measures.

Evaluating potential differences in the behavioral activation and inhibition systems between groups, nicotine users scored significantly higher than non-users on the Reward subscale of the Sensitivity to Punishment and Sensitivity to Reward Questionnaire (SPSRQ; $M_{nonuser}$ = 13.7, M_{user} = 16.3; $t(160) = -4.40$; $p = 0.0001$), which measures approach reactivity toward potentially rewarding situations. There were no significant differences between users and non-users on the Punishment subscale of the same questionnaire ($M_{nonuser}$ = 13.0, M_{user} = 12.1; $t(160) = 1.24$; $p = 0.22$), which assesses avoidance of situations involving possible aversive consequences (Torrubia et al., 2001). Users also scored significantly higher than nonusers on the Fun Seeking subscale of the Behavioral Activation and Behavioral Inhibition Questionnaire (BIS/BAS; $M_{nonuser}$ = 16.3, M_{user} = 17.3; $t(160) = -3.47$; $p = 0.001$), which measures the motivation to approach novel rewards (Carver & White, 1994). However, no significant differences were observed between users and non-users on the Behavioral Inhibition subscale of the same questionnaire ($M_{nonuser}$ = 27.0, M_{user} = 26.4; $t(160) = 1.36$; $p = 0.18$), which evaluates the motivation to avoid aversive outcomes. There were no significant differences between the nicotine and placebo groups on any measures evaluating the behavioral activation and inhibition systems. However, there was a trend for the nicotine group to score higher than the placebo group on the Reward Responsiveness subscale of the BIS/BAS ($M_{placebo}$ = 22.8, $M_{nicotine}$ = 23.7 ± 2.31; $t(59) = -2.32$, $p = 0.02$), which measures sensitivity to positive reinforcers (Carver & White, 1994).

Bivariate correlations were conducted between behavioral and physiological metrics of the three experimental tasks and the following questionnaires: FTND, SSS, Barratt Impulsiveness

Scale, SPSRQ, BIS/BAS, PANAS, and MNWS. Among nicotine users, the Barratt Attention subdomain of the Barratt Impulsiveness Scale was significantly negatively correlated with BART performance, where individuals with greater attentional instability had fewer balloon pumps that did not explode ($r(44) = -0.37$; $p = 0.01$). There was a trend for users who scored higher on the Disinhibition subscale of the SSS to have higher physiological responses to nicotine-associated images ($r(44) = 0.21$; $p = 0.02$). Individuals with greater levels of nicotine dependence as measured by the FTND rated the valence of affectively-positive images as more pleasurable ($r(78) = 0.31$; $p = 0.007$). Valence ratings of positive images were also significantly correlated with the Boredom Susceptibility subdomain of the SSS, where individuals less susceptible to boredom rated emotionally-positive images more favorably ($r(78) = -0.31$; $p = 0.004$). Finally, positive affect as measured by the PANAS was negatively correlated with valence ratings of nicotine images, where individuals with lower positive affect rated nicotine images as more pleasurable ($r(78) = -0.30$; $p = 0.008$). Positive affect was not significantly correlated with any other stimulus type.

Among non-users, valence ratings of facial attractiveness were significantly positively correlated with both the Behavioral Inhibition subscale of the BIS/BAS ($r(79) = 0.36$; $p = 0.01$) and with the Punishment subscale of the SPSRQ ($r(79) = 0.39$; $p = 0.0001$), indicating that individuals with greater motivation to avoid aversive outcomes rated the motivational value of facial stimuli higher. Moreover, non-users who scored higher on the Behavioral Inhibition scale also rated the valence of affectively-positive stimuli as more pleasurable ($r(79) = 0.37$; $p = 0.001$), and rated the valence of affectively-negative stimuli as less pleasurable ($r(79) = -0.2$; $p = 0.01$). Those who scored higher on the Reward Responsiveness subscale of the BIS/BAS also rated negative emotional stimuli as less pleasurable ($r(79) = 0.36$; $p = 0.001$). Impulsive non-

users who scored highly on the Disinhibition ($r(79) = 0.30$; $p = 0.007$) and Experience Seeking subscales ($r(79) = 0.28$; $p = 0.01$) of the SSS were more likely to positively rate the valence of nicotine images. Those who scored highly on the SPSRQ Reward subdomain were also more likely to rate nicotine images as more pleasurable ($r(79) = 0.36$; $p = 0.001$). Finally, non-users with greater Positive affective responses on the PANAS demonstrated enhanced skin conductance responses to positive ($r(57) = 0.32$; $p = 0.01$) and negative images ($r(57) = 0.35$; $p = 0.007$), with a trend to also more positively rate neutral ($r(57) = 0.31$; $p = 0.02$) and nicotine-related images ($r(57) = 0.30$; $p = 0.02$).

Examining nicotine-treated users, the Drive subscale of the BIS/BAS was significantly positively correlated with facial attractiveness ratings ($r(32) = 0.52$; $p = 0.002$), where individuals more likely to show approach behavior toward rewards were more likely to rate facial stimuli as attractive. Additionally, individuals in the nicotine group with higher scores on the Reward subscale of SPSRQ rated affectively-positive images as more positive ($r(31) = 0.47$; $p = 0.008$). Higher valence ratings of affectively-positive images were also positively correlated with scores on the Thrill and Adventure Seeking subscale of the SSS ($r(31) = 0.49$; $p = 0.005$), which relates to a desire for outdoor activities involving hyperstimulation and risk (Zuckerman et al., 2007). Higher reports of Negative affect on the PANAS was positively correlated with physiological responses to emotionally-negative stimuli ($r(29) = 0.48$; $p = 0.009$). Finally, there was a trend for nicotine-treated users with higher levels of nicotine dependence on the FTND to exhibit faster reaction times when rating the valence of positive images ($r(31) = 0.41$; $p = 0.02$).

Correlational analyses among placebo-treated users found a significant positive relationship between the Drive subscales of the BIS/BAS and arousal ratings of both affectively-positive ($r(26) = 0.58$; $p = 0.002$) and nicotine-related cues ($r(26) = 0.47$; $p = 0.01$). Placebo-treated users

who scored higher on the Punishment subdomain of the SPSRQ were more likely to rate emotionally-negative as more pleasurable ($r(26) = 0.47$; $p = 0.01$). Finally, positive correlations were detected among the MNWS and ratings of both affectively-positive and nicotine-related stimuli, where higher Withdrawal scores were correlated with higher ratings of positive ($r(26) = 0.47$; $p = 0.01$) and nicotine cues ($r(26) = 0.48$; $p = 0.01$).

3.4. Discussion

Evidence suggests that differential reward processing exists between nicotine users and non-users where nicotine users excessively attribute incentive salience to nicotine-associated stimuli relative to non-users. We therefore hypothesized that that the presentation of nicotine cues would result in higher skin conductance responses and enhanced ratings of subjective valence and arousal among nicotine users relative to non-users. In accordance with our hypothesis, we found that relative to non-users, nicotine users rated nicotine-associated images as significantly more pleasurable with a trend to also rate them more arousing. Compared to non-users, users also exhibited significantly greater SCRs when viewing nicotine-related images. These results support neuroimaging findings that show elevated mesocorticolimbic activity to drug cues (David et al., 2005; Franklin et al., 2007) and enhanced ratings of smoking-related images (Mucha et al., 1999; Mogg et al., 2003; Bradley et al., 2004, 2008) among nicotine users compared to non-users.

Of the few studies to examine differences in autonomic reactivity to smoking cues between smokers and non-smokers, Chae and colleagues showed that viewing smoking-related visual cues produced significantly larger pupil sizes in smokers compared to non-smokers. Smokers in their study also rated the smoking images with more pleasure and arousal than non-smokers (Chae et al., 2008). Together, these findings suggest that nicotine-associated cues

induce not only subjective emotional alteration but also sympathetic activation, key factors in modulating the motivational appetitive behavior associated with compulsive drug-taking and relapse. To our knowledge, the present study is the first to incorporate the International Affective Picture System and International Smoking Image Series to investigate differences in nicotine cue responsiveness between users and non-users using skin conductance.

Our hypothesis that nicotine users would assign enhanced motivational salience to nicotine-paired cues relative to neutral and affectively-negative cues was partially supported. While there were no differences in user valence ratings between nicotine and neutral images, users expressed enhanced arousal ratings and SCRs for nicotine images compared to neutral images. Users also rated the valence of nicotine-related stimuli as significantly more pleasant than negative affective images while arousal data indicated that users rated negative images more arousing than nicotine images; however, no differences in SCRs were observed between nicotine and negative image types. These findings suggest that subjective measures of valence and arousal may be dissociable from physiological skin conductance measures, each reflecting distinct properties of reward processing.

Generally, judgements of increasing pleasure and displeasure are positively correlated with arousal (Lang et al., 1997). Supporting this assertion, we found significant positive correlations between valence and arousal ratings for positive and nicotine stimuli, and a significant negative relationship between valence and arousal of negative images. It has also been suggested that reports of arousal signal the intensity of motivated behavior (Lang et al., 1997) where enhanced skin conductance responses to highly arousing pleasant and unpleasant images have been demonstrated (Lang et al., 1997; Bradley et al., 1992). In accordance with our hypothesis, nicotine cues elicited enhanced arousal ratings and SCRs relative to neutral cues.

Contrary to our hypothesis, however, nicotine cues were less arousing than negative cues and no differences in SCRs between the two image types were observed. This finding suggests that the magnitude of incentive salience attributed to nicotine rewards may be dependent upon affective stressors. For instance, Gilbert and colleagues (2008, 1995, 1997) suggest that nicotine only biases attention and associative processes toward rewarding cues to the degree that these cues are of equal salience. The affectively-negative cues used in our task were intentionally selected for their highly arousing and unpleasurable content to ensure robust responses. Therefore, it is possible that nicotine's incentive sensitizing effects toward drug rewards could not overcome the attentional and affect-inducing demands of the highly-salient negative stimuli.

Moreover, despite findings that skin conductance responses correlate positively with highly arousing pleasant and unpleasant images (Lang et al., 1997; Bradley et al., 1992), we found no significant correlations between arousal ratings and SCRs for any image type. Therefore, while subjective arousal and physiological measures may each reflect some aspect of appetitive motivation, our model suggests an uncoupling between these explicit and implicit measures. Others have also proposed a dissociation between explicit and implicit motivational systems in nicotine dependence (Cunningham et al., 2004; Sherman et al., 2003; Drobes & Tiffany, 1997). Implicit motives are thought to activate behavior toward desired affective experiences, irrespective of the individual's explicit intentions. Explicit motives, on the other hand, contain cognitive constructs about one's goals and depend on both the ability and willingness of an individual to assess and report these motives to the experimenter (McClelland et al., 1989; Schultheiss et al., 2009; Sherman et al., 2003). In this sense, implicit measures of motivated behavior, like physiological conductance responses, may be evoked without conscious awareness, while explicit self-report measures of arousal may reflect some level of introspection.

Additionally, discrete brain areas may be involved in the motivational processing of implicit and explicit information. For example, using neuroimaging to identify brain areas activated during the explicit or implicit emotional processing of facial expressions, Critchley et al., (2000) noted that explicit processing evoked significantly more activity in the temporal cortex than implicit processing, whereas implicit processing evoked significantly greater activity in the amygdala. Therefore, implicit and explicit measures may represent different underlying motivational processes that are subserved by distinct brain areas and may differentially influence addictive behaviors.

In addition to observing a dissociation between physiological responses and arousal, our analyses also indicated that SCRs did not correlate with valence ratings for any image type. Our findings support those of Lawn et al. (2015) who also found that the hedonic "liking" of rewards seems to be psychologically dissociable from motivated "wanting." They reported that dependent smokers made more reinforced responses for cigarette rewards than non-drug rewards on a computer-based operant task, but found no difference in hedonic pleasure ratings between any reward type. Behavioral and neurobiological distinctions between the hedonic "liking" and incentive "wanting" properties of reward stimuli have been previously reported. Chronic nicotine use is thought to sensitize mesocorticolimbic dopamine systems to excessively attribute incentive salience to drugs and drug-associated reward stimuli. This results in cue-triggered craving and consummatory behavior despite minimal or blunted pleasure following drug use (Robinson et al., 2015). Despite high prevalence rates of nicotine dependence, nonhuman self-administration rates for nicotine are low relative to other drugs of abuse (Caggiula et al., 2002) and nicotine produces little subjective euphoria in human users particularly after repeated use (Benowitz, 1996, 2009; West, 2009; Isomura et al., 2014), highlighting the limited role of hedonic pleasurability in

nicotine addiction.

Behaviorally dissociating pleasurable "liking" and motivated "wanting," one study used a dopamine transporter knockdown model to examine the effects of elevated dopamine on both the incentive motivation to obtain a sucrose reward and the affective liking elicited by the taste of sucrose (Peciña et al., 1997). They found that hyperdopaminergic mice exhibited greater incentive approach to acquire the sucrose reward but failed to elicit enhanced orofacial liking reactions to the sweet taste. In humans, extracellular dopamine enhancement by amphetamine increased both wanting and liking for the drug, however the increase in wanting was abolished by a dopamine antagonist without any apparent effect on liking (Brauer & de Wit, 1997). Similarly, smokers' cigarette consumption was attenuated by dopamine antagonist administration without reducing self-reported liking for cigarettes (Brauer et al., 2001).

Distinct neural substrates also seem to mediate components of "liking" and "wanting." While both systems are controlled by mesocorticolimbic structures, extracellular dopamine in the nucleus accumbens shell seems to confer hedonic properties, while extracellular dopamine in the accumbens core is responsible for motivated appetitive behavior (Robinson et al., 2015; Smith et al., 2011). Despite being behaviorally and biologically dissociable, both "liking" and "wanting" play an important role in initiating and maintaining nicotine dependence. Initial drug consumption is likely promoted by the expected euphoric ("liking") feelings produced by the drug. Subsequently, each drug encounter results in dopaminergic surges that sensitize the reward system to attribute enhanced incentive salience to the drug and its related cues. These cues then trigger motivated approach behavior ("wanting") to consume the drug. Since hedonic responses to nicotine predict future use (Pomerleau et al., 1993; Strong et al., 2011; Zabor et al., 2012), it is clinically relevant to assess how the "liking" and "wanting" systems work together to process

reward and contribute to nicotine dependence.

Some addiction theories based on incentive salience sensitization also propose that hyperreactivity to drug rewards among nicotine users reduces the attentional and motivational resources that remain for non-drug rewards (Diggs et al., 2013; Hogarth 2012; Hogarth & Chase, 2011, 2012; Lawn et al., 2015). For example, attenuated activity to non-drug rewards and positive hedonic stimuli have been reported in nicotine users relative to non-users (Martin-Soelch et al., 2003; Peters et al., 2011; Rose et al., 2012). Contrary to these findings, however, we found no differences between nicotine users and non-users on physiological responses or subjective valence and arousal ratings of affectively-positive stimuli. Moreover, we observed that positive images were rated as significantly *more* pleasurable and arousing than nicotine images, while SCRs were significantly greater to nicotine images relative to positive images. These results suggest that nicotine dependence is associated with hypersensitivity to nicotine rewards, however we found little evidence of hyposensitivity to non-drug rewards.

Other studies have also failed to show evidence of reduced motivation for non-drug rewards among nicotine users (Lawn et al., 2015 Bühler et al., 2010; Geier et al., 2000; Epstein et al., 1991). For example, using electroencephalographic (EEG) measures, Parker & Gilbert (2008) showed that relative to neutral images, both affectively-positive and nicotine-related images had significantly greater motivational significance to smokers. However, they found no difference in brain activity between positive and nicotine-related images, indicating that discrete measures may be sensitive to different aspects of incentive processing. Bühler et al. (2010) reported no differences in mesocorticolimbic activity to stimuli predicting monetary or cigarette rewards, nor were differences observed in the subsequent behavioral responses made to obtain these respective rewards in dependent and occasional smokers. In fact, in nondependent smokers,

higher neural reactivity and instrumental responses rates were observed for monetary rewards relative to cigarette rewards (Bühler et al., 2010). These results suggest a role for nicotine dependence in reward responding, therefore, future studies should selectively recruit individuals with greater indices of nicotine dependence to further characterize the relationship between nicotine dependence and the assignment of motivational valence to drug and non-drug rewards.

Epstein et al. (1991) also examined the effect of nicotine abstinence on the reinforcing value of money and cigarettes, and reported that when access to cigarettes was limited, deprived smokers initially responded more for cigarettes, but in later trials, were motivated equally by money and cigarettes. Non-deprived smokers, however, chose to work for money (Epstein et al., 1991). These results suggest that nicotine abstinence may polarize drug and non-drug reward values. However, while there was a trend for abstinent nicotine users in our sample to exhibit higher scores on the Minnesota Nicotine Withdrawal Scale (MNWS) relative to non-users, no significant correlations were found between the MNWS and subjective ratings or physiological responses for any image type among users. Given that our sample was primarily comprised of nondependent nicotine users it is unlikely that they were experiencing high levels of withdrawal. Therefore, future studies should selectively recruit individuals with greater nicotine dependence to better describe the relationship between nicotine deprivation and motivational processing.

In addition to examining differences between nicotine users and non-users, we subsequently administered nicotine or placebo to a separate group of nicotine-experienced participants to assess the influence of acute nicotine administration on drug and non-drug reward processing. Provided numerous studies indicating that acute nicotine enhances incentive salience and appetitive responding (Donny et al., 2003; Chaudri et al., 2006; Palmatier et al., 2006; Liu et al., 2007; Palmatier et al., 2007a, b; Caggiula et al., 2008), we hypothesized that relative to

placebo, nicotine would magnify the behavioral and physiological outcomes expected between users and non-users. However, contrary to our hypotheses, no significant differences were observed between the nicotine and placebo groups on any metrics: valence ratings, arousal ratings, or physiological measures of any stimulus type.

Our results contradict those of previous studies that suggest 12 hours of smoking abstinence attenuates self-reported affective valence produced by positive movie clips (Dawkins et al., 2007) while nicotine administration enhances positive reactivity to these clips (Dawkins & Powell, 2011). Dawkins et al. (2006) also reported that relative to abstinent users, users administered a 4mg nicotine lozenge displayed enhanced reward responsivity on a card-sorting task; however, this effect was only observed in heavier smokers who smoked 15 or more cigarettes daily. Interestingly, the same study found no difference between nicotine and placebo conditions on self-reported craving elicited by a smoking cue relative to a neutral cue again highlighting differences between explicit and implicit measures of reward responding. Additionally, while nicotine administration resulted in more errors color-naming positive words on an emotional Stroop task, Dawkins et al. (2006) found no differences between abstinent and satiated conditions on the error rate to color-name smoking-related words. Therefore, while nicotine administration may enhance the appetitive salience of pleasant words, grabbing attention and inhibiting smoker's ability to focus on other stimulus properties, nicotine did not seem to magnify salience of nicotine-associated words. This finding brings into question the specificity of nicotine's reinforcement enhancement for various types of stimuli.

These studies largely focus on nicotine's ability to attenuate abstinence-induced deficits in the brain reward system, suggesting that nicotine dependent individuals may be inherently hyposensitive to reward, predisposing them to seek nicotine to release dopamine and overcome

such deficits (Blum et al., 2000; Anselme et al., 2009). In the present study, we observed that withdrawal scores of placebo-treated participants were correlated with higher ratings of both affectively-positive and nicotine cues. However, we found no differences in withdrawal between the nicotine and placebo groups. Therefore, it is possible that users in our sample were not nicotine dependent enough to experience abstinence-induced deficits after 6 hours of forced abstinence, obscuring any differences between those in the placebo and nicotine conditions. It should be noted that models examining nicotine-induced reversal of withdrawal states provide different interpretation than those that propose acute nicotine produces an absolute enhancement of reward or reinforcement from other stimuli (Perkins et al., 2009).

As mentioned previously, and in a more direct test of nicotine's reinforcement enhancing effects, Perkins et al. (2009) assessed the effect of acute nicotine on operant responses for money, music, or the termination of aversive noise in nondependent smokers who would not experience withdrawal. Similar to our findings, Perkins found no effect of nicotine, administered by nasal spray or cigarettes, on responding for any reinforcer. Additionally, they found no influence of cigarette smoking on hedonic ratings of positive, negative, or neutral pictorial stimuli relative to denicotinized cigarette smoking or no smoking (Perkins et al., 2009). Therefore, nicotine's ability to enhance reward responding may be specific to nicotine dependent users and a function of withdrawal deficit reversal instead of reflecting reinforcement enhancement.

These conclusions may also support our finding that attractiveness ratings of facial stimuli were uninfluenced by nicotine use status and nicotine treatment, respectively, during the Facial Attractiveness Test. While numerous studies indicate enhanced activations of brain reward circuitry with increased judgements of facial attractiveness (Kampe et al., 2001; Cloutier et al.,

2008), only one group has previously examined the effects of nicotine on facial attractiveness ratings. Opposing our findings, Attwood and colleagues (2009, 2012) showed that nicotinized cigarettes increased attractiveness ratings of facial cues relative to denicotinized cigarettes. They found no difference in these effects between males and females, and observed no enhancement of subjective mood ratings, indicating that attractiveness ratings did not reflect global hedonic enhancement. However, like our study, their sample was comprised of nondependent smokers (average FTND = 0), and they observed no difference in cigarette cravings between conditions. This suggests that their effects were not a result of withdrawal relief and demands alternate theories as to why our results conflict.

Instead, methodological differences may account for discrepant findings between studies. While both studies used standardized photographic facial images, different image sets were used. While we selected images standardized as the most attractive and unattractive within the Chicago Face Database (Ma et al., 2015), it is possible that our images were less salient than those used by Attwood. Additionally, participants in the Attwood studies rated faces on a 7-point Likert scale, while we used a 5-point scale determined by the standardized image set we used. Therefore, it is possible that the 7-point Likert scale offered better discrimination. Different methods of nicotine administration also existed between studies. Attwood and colleagues used nicotinized and denicotinized cigarettes, while we used nicotine lozenges and similar-tasting placebo lozenges. The method of nicotine administration does seem to play an important role in moderating reward responsivity where reinforced responses for different reward types have shown to be dependent on the route and dose of nicotine administration (see section *2.4. Discussion).* Thus, additional research is needed to examine the effects of alternate methods of nicotine consumption on facial attractiveness reward enhancement.

In addition to characterizing the role of nicotine in the assignment of motivational valence to nicotine and non-nicotine rewards, the present study also aimed to identify potential correlates between impulsivity and reward responding. In accordance with abundant evidence that suggests personality traits associated with impulsivity confer vulnerability to nicotine dependence (Lovic et al., 2011; Bickel et al., 1999, 2008; Doran et al., 2004; Mitchell, 1999, 2004; Perkins et al., 2008), our questionnaire measures indicated that nicotine users scored significantly higher than non-users on subscales of the Sensation Seeking Scale (SSS) that are associated with greater risk of participating in drug use, alcohol use, vandalism, and/or unsafe sex, and an increased pursuit of an unconventional lifestyle. Nicotine users also scored higher than non-users on subscales of the Barratt Impulsiveness Scale demonstrating greater tendency to act without thinking. There were no differences between nicotine- and placebo-treated users on any impulsivity questionnaire measures, suggesting that impulsive characteristics may be inherent traits that predicate nicotine use, and are less influenced by situational manipulations.

Given evidence that drug users may derive greater reinforcement from drug use than non-users (Lovic et al., 2011; Doran et al., 2007; Cascella et al., 1994) and may be more affected by delays to reinforcement (Sweitzer et al., 2008; Baker et al., 2003; Bickel et al., 1999, 2008; Mitchell, 1999, 2004; Wilson et al., 2015), we hypothesized that nicotine users would make riskier choices than non-users on the Balloon Analogue Risk Task (BART). We observed that individuals with greater attentional instability (as evidence by the Barratt Impulsiveness Scale) had fewer balloon pumps that did not explode, suggesting some relationship between self-report measures of impulsivity and the BART task. However, we found no differences between nicotine users and non-users on any BART measure, regardless of whether participants were working for virtual rewards or had the potential to earn real monetary rewards by working for $20 Amazon

gift card lottery entries. Moreover, nicotine administration did not influence BART measures relative to placebo. We also found no relationship between BART measures and physiological responses or subjective ratings made during the Drug/Non-Drug Responsiveness Task or the Facial Attractiveness Test.

These results contradict Lequez et al. (2003) who demonstrated that smokers scored significantly higher than non-smokers on the BART in terms of monetary earnings, adjusted average number of balloon pumps, total number of balloon pumps, and explosions. While they conducted the task using 30 balloons, they reported almost identical results within the first 10 balloons, providing justification for our use of 10 balloon trials. Importantly, Lequez administered real money at the end of the experiment session based on the participant's performance. By attempting to replicate these findings using virtual earnings with no financial payout, or with entries into a lottery for a possible payout, it is possible that the reinforcers of the present study were not salient enough to generate robust responses. In support of this theory, Palmatier et al. (2007) demonstrated that the reinforcement enhancing effects of nicotine are more robust for stimuli with moderate reinforcing value compared to weaker reinforcers. In this study, rats were administered either nicotine or saline, and were then trained to lever press for one of two visual stimuli that varied in their reinforcing value. Specifically, the more reinforcing stimulus involved the removal of an aversive white house light, while the lesser reinforcing stimulus involved the presentation of a red house light. They found that the reinforcement enhancing effects of nicotine were more robust for the more reinforcing removal of the house light, relative to the weaker reinforcing presentation of the red light. They therefore concluded that the reinforcement enhancing action of nicotine depends on the salience of the primary reinforcer.

However, Cavalca et al. (2013) also failed to find an effect of smoking status on the total number of balloon pumps when administering real money to participants following BART performance, suggesting that the reinforcing value of the task may not be the only explanation for discrepancies between results. Interestingly, in the only other study to investigate BART responses for non-redeemable earnings, a significant negative relationship between BART performance and nicotine dependence was found where greater risk-taking propensity was associated with lower levels of dependence (Ryan et al., 2013). We, however, did not find correlations between nicotine dependence and any BART measure, though this may have been due to little variance in nicotine dependence among our sample.

Finally, examining potential correlates between self-report measures of impulsivity and reward responding, we only observed a trend for users who scored higher on the Disinhibition subscale of the SSS to have higher physiological responses to nicotine-associated images, suggesting only a weak relationship between impulsivity and nicotine cue reactivity in this study. Given that others have identified an association between impulsive personality characteristics and smoking onset (Burke et al., 2001, Burt et al., 2000; Harakeh et al., 2006; Masse & Tremblay, 1997), as well as relapse rates (Krishnan-Sarin et al., 2007; Diergaarde et al., 2008), it is critical to investigate the relationship between impulsive traits and reward processing to inform etiological models and treatment interventions. Moreover, future studies should explore how the magnitude of reward saliency influences BART task responses.

In conclusion, the present findings extend previous research by examining the magnitude of incentive salience devoted to nicotine and non-nicotine rewards. Our findings demonstrate that nicotine users attribute enhanced incentive salience to nicotine-related rewards relative to non-users. However, we observed little evidence of hyporeactivity to non-nicotine rewards among

nicotine users. Moreover, acute nicotine administration did not influence motivational processing in our nondependent sample. Our results suggest that nicotine's ability to enhance reward responding may be specific to nicotine dependent users and a function of withdrawal deficit reversal instead of reflecting reinforcement enhancement. Therefore, the greatest limitation to the present study is that our sample lacked diversity in nicotine dependence levels. Future studies should selectively recruit groups based on nicotine dependence levels to better characterize the relationship nicotine dependence and motivational reward processing. Additionally, further investigation is needed to determine how impulsive personality characteristics moderate responsiveness to reward.

IV. THE EFFECT OF ACUTE NICOTINE ADMINISTRATION ON HUMAN DELAY AND TRACE FEAR CONDITIONING

4.1. Introduction

In addition to enhancing the incentive properties of rewarding stimuli via Pavlovian conditioning mechanisms, nonhuman studies suggest that nicotine may also enhance the motivational value attributed to aversive cues. Aberrant conditioning mechanisms to aversive stimuli have been implicated in the development of anxiety disorders (AD), like generalized anxiety disorder, panic disorder, phobias, and post-traumatic stress disorder (PTSD), where learned anxiety resulting from trauma may be generalized to similar but neutral cues or contexts (Morrow et al., 2011; Bush et al., 2007). Epidemiological studies estimate that the lifetime prevalence of AD, is between 15-34% among U.S. adults (Bandelow & Michaelis, 2015), suggesting that AD are one of the most common mental health disorders (Kessler et al., 2012).

Notably, there is striking comorbidity between AD and nicotine dependence (Breslau et al., 2003, 2004; Feldner et al., 2007). According to a comprehensive summary by Kutlu & Gould (2015) describing the relationship between disorders, nicotine dependence is more prevalent

among individuals with AD (43.5%) relative to the nonclinical population (22.5%; Lasser et al., 2000; Ziedonis et al., 2008). Conversely, prevalence rates of AD are higher among smokers (22%) than non-smokers (11.1%; Grant et al., 2006). Consistent with indications of high rates of dependence among individuals with AD, nicotine use is positively correlated with vulnerability to develop PTSD (Koenen et al., 2005) and PTSD symptom severity (Thorndike et al., 2006; Baschnagel et al., 2008). Moreover, individuals with PTSD demonstrate greater difficulty maintaining nicotine abstinence (Lasser et al., 2000; Hapke et al., 2005) and are more likely to relapse following cessation attempts (Beckham et al., 2012). A bidirectional relationship between nicotine dependence and AD has also been identified in individuals with panic disorders (Cosci et al., 2010; Goodwin et al., 2005), phobias (Schumann et al., 2004; Sonntag et al., 2000), and generalized anxiety disorder (Moylan et al., 2012). Therefore, while nicotine use may attenuate symptoms of anxiety short-term (Robinson et al., 2009), it may also predispose individuals to AD by enhancing baseline symptom severity over time (Kutlu & Gould, 2015; Kutlu et al., 2015).

Although different AD are characterized by distinct symptoms, exaggerated fear responses are common across AD subtypes (Lissek et al., 2005; Shin & Liberzon, 2009). For example, fearful memories, nightmares, and flashbacks are common among individuals with PTSD and manifest as physiological distress and hyperarousal (Gualtieri & Morgan, 2008; Kutlu & Gould, 2015). Excessive fear responses to specific objects or situations are also common among individuals with panic disorder and specific phobias (Lissek et al., 2009; Öhman & Mineka, 2001). Given that excessive fear is a central component of AD, Pavlovian fear conditioning (FC) is a commonly used translational model of the fear responses that may be relevant to some AD (Shin & Liberzon, 2009; Davis, 1992; Fendt & Fanselow, 1999; LeDoux,

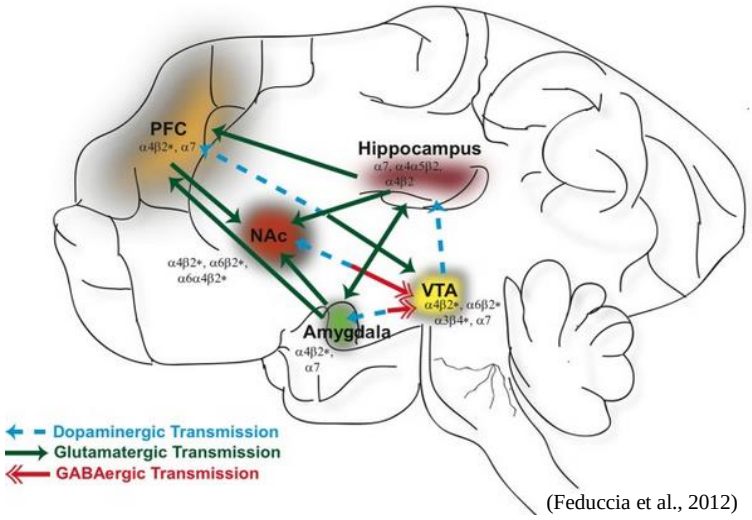
2000; Maren, 2001). The FC paradigm involves repeatedly pairing a neutral conditioned stimulus (CS; i.e. a light cue) with an aversive unconditioned stimulus (US; i.e. a shock). After repeated pairings, the CS alone comes to elicit a conditioned fear response. Conditioned fear responses can be elicited by exposing the subject to the conditioned cue that was associated with the aversive stimulus, or to the context in which the aversive stimulus was experienced (Blanchard & Blanchard, 1972; Bolles & Fanselow, 1980; LeDoux, 1987). In nonhumans, indices of fear are often measured using behavioral measures like freezing (inhibition of movement) or enhanced startle. In humans, fear is often quantified using psychophysiological responses, like skin conductance response (SCR), heart rate, facial EMG, or pupillometry (Adolphs, 2014); however, SCR is the most widely used psychophysiological measure in human FC studies (VanElzakker et al., 2014).

Importantly, the neural underpinnings that mediate fear responding are analogous across species (LeDoux, 1996; Delgado et al., 2006). Evidence of both amygdala and hippocampal involvement in the learned association between context and unconditioned stimulus have been reported in human (see review: Davis & Whalen, 2001; Cheng et al., 2003; Alvarez et al., 2008; Knight et al., 2004) and nonhuman studies (Phillips & LeDoux, 1992; Rudy et al., 2004; Paré et al., 2004). Conversely, studies across species suggest that forming an association between the cued conditioned stimulus and the unconditioned stimulus depends on the amygdala, but does not require hippocampal involvement (see review: Maren et al., 2013; Sanders et al., 2003; LaBar et al., 1998; Bechara et al., 1995; Davis et al., 1992; LeDoux et al., 2003; Marschner et al., 2008).

Notably, dysregulations of these brain regions have been reported among individuals with AD, substantiating the use of the FC model to provide insight into factors that contribute to AD

development (see review: Martin et al., 2009; Bremner et al., 2005; Werner et al., 2009). For example, relative to the nonclinical population, hyperactivation of the amygdala is observed in patients with social anxiety in response to angry facial stimuli (Evans et al., 2008), and in patients with specific phobia in response to phobia-relevant stimuli (Wendt et al., 2008). Moreover, hippocampal volumes are reportedly diminished in individuals with PTSD (Villarreal et al., 2002; Bossini et al., 2008; Smith et al., 2005), and are inversely associated with PTSD symptom severity (Gilbertson et al., 2002; Bremner et al., 2003). Neuroimaging studies also report overlap between the neural substrates that underlie AD and those of nicotine dependence. For example, Due et al. (2002) reported that relative to non-smokers, smokers exhibited enhanced brain activation in the striatum, as well as the amygdala and hippocampus following exposure to smoking cues. Therefore, the ability of nicotine to alter the neural systems underlying AD may partially explain the relationship between disorders.

Implications of nicotine receptor modulation on the progression and expression of AD have been extensively investigated in nonhuman FC studies. In numerous fear conditioning experiments, acute nicotine enhanced hippocampal-dependent contextual fear conditioning, but not hippocampal-independent cued conditioning (Gould & Wehner, 1999; Gould & Higgins, 2003; Wehner et al., 2004; Davis et al., 2006b, 2007, Gould & Lommock, 2003, Gulick &



(Feduccia et al., 2012)

Figure 9. *Neuronal nicotinic receptors in the human brain.*

Gould, 2008, Portugal et al., 2012; Figure 9). For example, in a study by Gould & Wehner (1999), mice were injected with either nicotine or saline prior to undergoing a training session during which baseline freezing behavior was established for the first 120 seconds, and then a 30-second auditory CS was presented. During the last two seconds of the CS, a shock was administered, and freezing was scored before the next CS-US pairing 120-seconds later. Twenty-four hours after training, mice were again administered either nicotine or saline and tested in the original chamber for fear to the context for five minutes. One hour later, the mice were placed in a novel context where baseline freezing was measured for 3 minutes, followed by 3 minutes of measured freezing to the auditory CS. They found nicotine enhanced contextual learning as evidenced by increased freezing to the shock-paired context relative to the saline group. Interestingly, this effect was only observed when nicotine was administered during both training and testing days, and not when nicotine was given only on training day, or only on testing day. This effect has been reproduced by several other studies that also report the requirement of nicotine administration on both days to produce enhanced context conditioning (Gould, 2003; Gould & Higgins, 2003; Gould & Lommock, 2003). Moreover, no nicotine enhancement of hippocampal-independent cued fear learning of the auditory CS-US association was found. In a subsequent study, the same group demonstrated that the lack of effect on cued conditioning was not a result of ceiling effects since reducing the number of CS-US pairings still demonstrated no enhancement of cued FC (Gould et al., 2004).

These findings suggest that nicotine facilitates processes that encourage both acquisition and subsequent access of the learned response. Therefore, nicotine may potentiate contextual fear learning by promoting attention and/or multimodal processing (Gould & Wehner, 1999). In this sense, nicotine's effects on learning may be limited to more complex tasks that demand

enhanced attention to a multitude of environmental cues, like contextual fear conditioning, instead of simpler tasks like cued learning. Interestingly, while the presence of nicotine was needed at both the initial acquisition and access of the context fear memory 24-hours later, enhanced contextual FC persisted in the absence of nicotine administration at a 1-week retest (Gould & Higgins, 2003). Therefore, while nicotine enhances hippocampal-mediated learning, once the enhanced memory is committed to long-term storage, nicotine is no longer needed to access it. This indicates that the activation of nicotinic acetyl choline receptors likely play a role in early learning processes, but different systems mediate access to enhanced memories once they are learned (Gould & Higgins, 2003).

To elucidate whether nicotine enhances contextual fear conditioning by strengthening the context-shock association, or strengthening learning of the context itself, Kenney & Gould (2008) employed a paradigm using the context pre-exposure faciliatory effect (CPFE) to dissociate these forms of learning. This model is based on findings from Fanselow (1990) demonstrating that an animal will show negligible fear to a context if shocked immediately upon being placed in that context. However, if the animal sufficiently explores the context prior to conditioning the next day (context pre-exposure), they exhibit significantly more conditioning following immediate shock. Recognizing that context learning and CS-US associative learning occur on separate days, Kenney & Gould (2008) used CPFE to determine which phase of contextual fear conditioning is modulated by nicotine. They found that nicotine administered prior to both pre-exposure and testing significantly enhanced contextual conditioning, but nicotine administration prior to immediate shock had no effect. Moreover, nicotine had no effect when only administered prior to pre-exposure, immediate shock, or testing. They therefore concluded that nicotine specifically enhances learning of the context.

Interestingly, different neural substrates seem to underlie context conditioning and CS-US conditioning. Communication between the hippocampus and cortex is thought to mediate context learning, while interactions between the hippocampus and amygdala may facilitate context-shock associative learning (Kenney & Gould, 2008; Fanselow, 2000; Matus-Amat et al., 2004; Rudy et al., 2004). Since nicotine failed to enhance context-shock learning, nicotine likely does not strengthen activations within the hippocampus-amygdala pathway. Instead, nicotine seems to specifically enhance activity between the hippocampus and cortex, or even within the hippocampus itself. This second hypothesis is supported by evidence that direct infusion of acute nicotine into the dorsal hippocampus enhanced context learning, while cortical infusions had no significant effect on conditioning (Davis et al., 2007; Kenney et al., 2012).

Clearly, nicotine preferentially enhances hippocampal-dependent versions of fear conditioning given demonstrations of nicotine-enhanced context conditioning, but not delay cued conditioning. However, cued FC parameters can be modified to recruit the hippocampus if a temporal delay, during which no stimulus is presented, is inserted between the offset of the cue and the onset of the US (Gould et al., 2004; Crestani et al., 2002; McEchron et al., 1998; Quinn et al., 2002). This type of hippocampal-dependent learning is called trace fear conditioning and has also been shown to be enhanced by nicotine (Gould et al., 2004; Raybuck & Gould, 2009; Davis et al., 2006b). In one study utilizing a trace FC procedure, freezing was measured during a 120-second baseline period preceding the presentation of an auditory CS (Gould et al., 2004). The CS was then presented for 15 seconds and was followed by a 30-second trace period in the absence of any CS or US presentation. A 2 second shock US was administered after the trace period. The researchers found that nicotine administered prior to training and testing enhanced trace fear conditioning as evidenced by increased freezing to the CS by nicotine-treated mice

relative to saline-treated mice. Moreover, this finding of nicotine-enhanced trace conditioning has been replicated by several other nonhuman studies (Davis & Gould, 2006; Raybuck & Gould, 2009).

There are a variety of methods used to examine whether human participants are consciously aware of the predictive association between CS and US, often reported in human studies via dichotomous forced choice tests, visual analog scales, and Likert scales (Boddez et al., 2013). This operationalization is known as contingency awareness (Carter et al., 2006) and is believed to affect some forms of conditioning. For example, several human studies suggest that contingency awareness is required for successful trace conditioning where individuals who fail to show knowledge of the CS-US contingency do not exhibit trace conditioning (Clark & Squire, 1998; Manns et al., 2000a, 2000b; Clark et al., 2001; Knight et al., 2006; Weike et al., 2007; Klucken et al., 2009). Delay cued FC, on the other hand, does not seem to necessitate contingency awareness as it has been demonstrated in individuals who were both aware and unaware of the CS-US relationship (Clark & Squire, 1998; Clark et al., 2001; Manns et al., 2001; Smith et al., 2005; Knight et al., 2006; Weike et al., 2007). No studies to our knowledge have examined the interaction between nicotine and contingency awareness on fear conditioning. Since contingency awareness may facilitate CS-US associative learning, further exploration of this factor is critical for comprehensive understanding and treatment of AD.

Summary: In sum, the results from these studies suggest that acute nicotine enhances hippocampal-dependent contextual and trace variants of FC but has no effect on hippocampal-independent delay cued FC. To our knowledge, studies examining the effects of nicotine on learned fear have been exclusively limited to nonhumans. Therefore, more research is needed to address whether nicotine also enhances fear learning in humans, with specific enhancement of

hippocampal-dependent FC. We hypothesize that when compared to placebo treatment, a 2mg nicotine lozenge administered prior to conditioning will enhance hippocampal-dependent contextual fear, as measured by skin conductance response, and will have no effect on hippocampal-independent cued fear. In a serially conducted experiment, we examined the effect of nicotine on trace fear conditioning. We hypothesized that a 2mg nicotine lozenge administered prior to conditioning would significantly enhance trace conditioned fear relative to placebo, as measured by skin conductance response. Moreover, we investigated the effect of contingency awareness on nicotine-enhanced trace FC.

Participants also completed several questionnaires querying nicotine dependence and indices of stress and anxiety. These questionnaires included The Fagerstrom Test for Nicotine Dependence (FTND; Fagerstrom, 1989), the Depression Anxiety Stress Scales (DASS; Lovibond & Lovibond, 1995), and the State Trait Anxiety Inventory (STAI; Spielberger et al., 1983). As mentioned previously, the FTND is one of the most widely used, standardized instruments for assessing the intensity of physical dependence to nicotine. We hypothesized that individuals more dependent on nicotine would demonstrate greater nicotine enhanced contextual and trace fear learning since their neural circuitry may be more readily primed for nicotine's effects on learning. The DASS is a 42-item instrument designed to differentially measure the negative emotional states of depression, anxiety, and stress, while the STAI is a 40-item self-report measure that indicates the intensity of anxious feelings and distinguishes between state anxiety (a temporary condition experienced in specific situations) and trait anxiety (a general tendency to perceive situations as threatening). Evidence suggests a bidirectional relationship between nicotine dependence and anxiety where nicotine use may attenuate symptoms of anxiety short-term (Robinson et al., 2009), but also may also predispose individuals to AD by enhancing

baseline symptom severity over time. Therefore, it is of interest to investigate whether individuals with greater indices of stress and anxiety are more likely to demonstrate enhanced fear conditioning.

4.2. Material and Methods

4.2.1. Participants: University of Connecticut undergraduates 18 years of age or older were recruited from introductory psychology classes. All participants were pre-existing nicotine users with varied levels of dependence; however, we did not differentially recruit high and low nicotine users. Nicotine-naïve participants were not used for the study as they are more likely to experience adverse side effects of nicotine administration. Participants were instructed to abstain from eating and from using nicotine for at least six hours prior to the experiment. Participants with cardiac conditions, neurological conditions, and those who were pregnant were ineligible to complete the study. Participants received class credit or gift card compensation for their participation.

4.2.2. Apparatus: A 17-inch IBM-compatible computer with a SVGA color monitor was used for testing. Physiological measurements were recorded using a Biopac Systems MP150 data acquisition system. The Biopac MP150 system was connected via an Ethernet cord to a laptop running Biopac Acqknowledge software, version 4.2.1. The Biopac MP150 system received digital TTL signals through its isolated digital interface connecting to the parallel port on the stimulus computer running the VR software E-prime. Electrodermal activity (EDA) was collected continuously from two disposable electrodes on the index and middle fingers on the participant's non-dominant hand.

4.2.3. Procedure: This was a one-day, one-hour experiment. Participants arrived after at least 6 hours of nicotine abstinence, and consent was obtained. To ensure tobacco abstinence,

breath samples were obtained using a CoVita Smokerlyzer carbon monoxide sensor. Participants with a CO reading of PPM > 10 were excluded from analysis (Perkins et al., 2012). Participants with CO readings of PPM > 10 were rescheduled (Perkins et al., 2012). To note, CO readings would only be detected in individuals who smoke cigarettes or cigars, and not those who use other methods of nicotine consumption (i.e. e-cigarette, chewing tobacco, etc.). We did not have the means to record plasma nicotine levels to confirm use of other nicotine products. Participants were then randomly assigned to receive either a 2mg nicotine lozenge or a similar-tasting placebo (Altoids Wintergreen mint see section *2.3.3. Procedure* for dose rationale and administration details). While the lozenge dissolved, participants completed several questionnaires. A general questionnaire was administered to query participants basic demographics, as well as their nicotine use history, including questions about when they last used nicotine, their frequency of nicotine use, and which nicotine-containing products they prefer. Participants also completed the Fagerstrom Test for Nicotine Dependence (FTND; Fagerstrom, 1989), the Depression Anxiety Stress Scales (DASS; Lovibond & Lovibond, 1995), and the State Trait Anxiety Inventory (STAI; Spielberger et al., 1983).

Following completion of the questionnaires, electrodermal electrodes were placed by the experimenter on the index finger and middle finger of the participant's non-dominant hand, and stimulus electrodes were placed on the underside of the subject's non-dominant forearm. Shock intensity was determined by the participant. To do this, the STIMSOC adapter was set to the 200V setting, and a VR session was initiated in a barren, never-to-be-seen again virtual environment. Voltage was incrementally increased by the experimenter until the participant indicated that a shock intensity level had been reached that was aversive, but not painful, and this intensity setting was used for the US in all subsequent fear conditioning trials.

4.2.3.1 Delay Cued and Contextual Fear Conditioning Procedure

Acquisition session- Following the physiology set-up, participants were provided instructions about the fear conditioning procedure. Some studies report that CS-US contingency awareness, commonly operationalized via dichotomous forced choice tests, visual analog scales, and Likert scales (Boddez et al., 2013), is an important determinant of fear conditioning (Lovibond & Shanks, 2002; Klucken et al., 2009; Tabbert et al., 2006). Therefore, participants were told "One of the lights presented on the computer screen will be followed by a shock, the other will not. You will be able to figure out which is which by paying close attention." Participants then completed two, 260-second conditioning acquisition session while confined to Room A of the virtual environment, since our lab has previously reported that two acquisition sessions results in successful differential fear conditioning (Errante, 2017). The environment consisted of two visually-distinct rooms that were identical in shape and size, but differed in colors, patterns, and existing items (see Figure 1 in section *2.2.3*). Participants were conditioned to the onset of a green floodlight (CS+) presented for 8s. The CS+ was partially reinforced by a 500ms shock that occurred 7.5s following the onset of the CS+. Specifically, five of the six CS+ presentations were reinforced since hippocampal activation has been associated with uncertainty of receiving an aversive stimulus (Ploghaus et al., 2003). An 8s red flood light served as the CS- during which no shock occurred. Conditioned stimuli were presented six times each, for a total of twelve stimulus presentations, with an inter-stimulus interval of 20s ± 4s. Five presentations of the CS+ were reinforced with an aversive shock, and one was not, since studies have shown that some brain regions, including the prefrontal cortex and hippocampus, activate more strongly under uncertain conditions (Critchley et al., 2001; Dunsmoor et al., 2007).

Contextual test session- A 100-second test session was used to test for contextual fear.

The first 20s of the context test began in a barren VR room to establish baseline physiology. Participants were then transported within the test session to either Room A (the previously shock-paired room) or Room B (the novel, neutral, shock-free safe room). Twenty seconds later, participants were transported back to the barren VR room. Following another twenty seconds, participants were transported to either Room A or Room B, such that if they had previously visited Room A, they would instead be transported to Room B, or vice versa. Finally, participants returned to the barren VR room for the remaining 20s. No floodlight cues nor aversive shocks were presented during the context test session. The order in which participants visited Room A and Room B were counterbalanced.

Delay cued test session- To test for conditioned fear to the light cues, participants underwent a 120-second cued fear test session. The entirety of this session occurred in Room B, where green (CS+) and red (CS-) flood lights were pseudo-randomly presented twice in 8s durations. Twenty second inter-stimulus intervals separated the floodlight onsets. No aversive shocks were presented during the cued test session. The order in which participants completed the context test session and cued test session was counterbalanced.

Following all three virtual sessions, participants completed a five-item questionnaire to assess their awareness of the CS-US contingencies (modified from Clark & Squire, 1999). Using true or false responses, participants were asked to identify which light predicted the shock, and the order in which the light and shock occurred (i.e. “I believe the shock usually occurred AFTER the green light.”) Finally, the Simulator Sickness Questionnaire (Kennedy et al., 1993) was administered to address potential adverse side effects associated with navigating virtual environments, including fatigue, boredom, headache, nausea, faintness, confusion, etc. Participants who indicated that they had experienced any adverse symptoms were also asked to

indicate symptom severity (slight, moderate, or severe). This measure was used as a covariate in exploratory analysis to ensure that nausea did not interfere with fear learning.

4.2.3.2. Trace Fear Conditioning Procedure

Acquisition session- Following the physiology set-up and administration of task instructions, participants completed one, 260-second conditioning acquisition session in the virtual environment. The environment consisted of the same two visually-distinct rooms as in the *Delay Cued and Contextual FC* procedure (Figure 1). The acquisition session occurred in Room A, where participants were conditioned to the onset of a green floodlight (CS+) presented for 8s. However, a delay period was inserted between the light cue and shock, such that the CS+ was partially reinforced by a 500ms shock that occurred 2.5s following the offset of the CS+. A 4.5s delay period was chosen because imaging studies examining neural activity during trace FC in humans have reported hippocampus activations for trace intervals lasting 1s, (Büchel, 1999), 4.5s (Knight et al., 2006), and 10s (Knight et al., 2004), where 4.5s is a moderate, but effective, delay. As in the *Delay Cued and Contextual FC* procedure, an 8s red flood light served as the CS- during which no shock occurred. Conditioned stimuli were presented six times each, for a total of twelve stimulus presentations, with an inter-stimulus interval of 20s ± 4s. Five presentations of the CS+ were reinforced with an aversive shock, and one was not.

Cued and Contextual test sessions: Both the context test session and cued test session were identical to the *Delay Cued and Contextual FC* procedure, and again, the order in which participants completed the context test session and cued test session was counterbalanced. Participants also completed the same post-test questionnaires as in the *Delay Cued* experiment.

4.3. Results

4.3.1. Statistical Analysis: Electrodermal activity data were visually inspected and corrected for artifacts before statistical analysis. Specifically, data were resampled to 62.5 samples/second and median value baseline smoothing was applied to eliminate rapid transient events. To correct for high frequency electrical noise and movement artifacts, a low pass filter optimized for the sample rate cutoff was applied, per standard pre-processing techniques (Braithwaite et al., 2013) The change in skin conductance response (SCR) was calculated for each trial by subtracting the mean baseline skin conductance level during the 1s immediately prior to CS onset from the maximum skin conductance level recorded 3-8s following stimulus onset. This method of scoring SCR data as a second interval response (SIR) has been validated across numerous human fear conditioning physiological publications (see review Lonsdorf et al., 2017) and allows for the detection of the maximal increase in SCRs during the CS presentation. The SIR is generally considered an emotional response, elicited by anticipation of the US, and reflects learning the CS–US association (Boucsein, 2012; Prokasy and Kumpfer, 1973; Wolter and Lachnit, 1993). Participants who failed to exhibit positive response magnitudes (greater than 0) in reaction to the US during the SIR were excluded from analysis. Statistical analyses were conducted with analyses of variance (ANOVAs) with repeated measures and t-tests. A significance level of $p = 0.05$ was used in all analyses. Greenhouse–Geisser corrections were used for main effects and interactions involving factors with more than two levels to correct for violations of sphericity.

4.3.2. Delay Cued and Contextual Fear Conditioning: Of the eighty-four undergraduates recruited for this study, data from seventeen participants were excluded due to equipment malfunction ($n = 2$), participant failure to follow directions ($n = 2$), poor physiology recordings

resulting from weak electrode adhesion or other recording artifacts (n = 6), or a lack of SCR response to the US as defined by failure to exhibit positive response magnitudes (greater than 0) to the US during the SIR (n = 7). Significantly more placebo-treated participants were lost in attrition than nicotine-treated participants (4 (4.8%) in the nicotine group and 13 (15.4%) in the placebo group; $p < 0.05$). Sixty-seven participants were included in the final analysis ($n_{nicotine}$ = 29, 10 female, average age = 19.3 ± 1.46; $n_{placebo}$ = 38, 12 female, average age = 19.3 ± 1.22). There were no significant differences between treatment groups in age ($t(62) = 0.27$, $p = 0.79$), hours since last nicotine use ($M_{nicotine}$ = 14.0 ± 10.5, $M_{placebo}$ = 11.7 ± 8.28; $t(62) = 0.99$, p = 0.32), or weekly nicotine consumption ($M_{nicotine}$ = 30.2 ± 42.0 $M_{placebo}$ = 18.7 ± 18.6; $t(62) = 1.47$, $p = 0.15$). Additionally, there were no treatment differences on any pre-test questionnaire measures (Table 13): Fagerstrom ($M_{nicotine}$ = 2.93 ± 2.70, $M_{placebo}$ = 2.97 ± 2.70; $t(62) = -0.06$, p = 0.95), DASS Stress ($M_{nicotine}$ = 7.93 ± 7.29, $M_{placebo}$ = 6.94 ± 6.15; $t(62) = 0.59$, $p = 0.56$), DASS Anxiety ($M_{nicotine}$ = 8.36 ± 7.14, $M_{placebo}$ = 6.83 ± 6.56; $t(62) = 0.89$, $p = 0.38$), DASS Depression ($M_{nicotine}$ = 5.50 ± 5.77, $M_{placebo}$ = 6.22 ± 8.79; $t(62) = -0.38$, $p = 0.71$), STAI ($M_{nicotine}$ = 27.5 ± 10.7, $M_{placebo}$ = 27.6 ± 10.6; $t(62) = -0.06$, $p = 0.96$). However, the nicotine group scored significantly higher than placebo group on the Nausea subscale of the post-test Simulator Sickness Questionnaire ($M_{nicotine}$ = 4.00 ± 3.04, $M_{placebo}$ = 2.65 ± 2.31; $t(62) = 2.04$, $p = 0.05$; Table 13). Therefore, nausea was used as a covariate in exploratory analyses.

Questionnaire Measure	Users (M ± SD)	Non-users (M ± SD)	*t*	*df*	*p*
Fagerstrom	2.93 ± 2.70	2.97 ± 2.70	-0.06	62	0.95
DASS Stress	7.93 ± 7.29	6.94 ± 6.15	0.59	62	0.56
DASS Anxiety	8.36 ± 7.14	6.83 ± 6.56	0.89	62	0.38
DASS Depression	5.50 ± 5.77	6.22 ± 8.79	-0.38	62	0.71
STAI	27.5 ± 10.7	27.6 ± 10.6	-0.06	62	0.96
Nausea	4.00 ± 3.04	2.65 ± 2.31	2.04	62	0.05

Table 13. *No significant differences between nicotine use status on pre-test questionnaire measures. Nicotine users scored significantly higher than non-users on post-test nausea measure ($t(62) = 2.04$, $p = 0.05$).*

Acquisition session: To examine the effect of treatment on the acquisition of conditioned fear across both acquisition sessions, SCR data were analyzed using a 2-factor Trial (1-12) X Stimulus Type (CS+, CS-) repeated measures ANOVA with Treatment (nicotine, placebo) as the between-subjects factor. Analysis yielded significant Stimulus Type ($F(1, 65) = 12.8$, $p = 0.001$) and Trial main effects ($F(5.88, 382.0) = 4.86$, $p < 0.0001$). As expected, there was a larger mean SCR magnitude to the CS+ (M = 0.32 ± 0.34) compared to the CS- (M = 0.20 ± 0.34), demonstrating conditioned fear responding. Adjusting alpha to 0.01 to account for multiple tests, a series of paired t-tests comparing SCR magnitude for CS+ versus CS- trial pairings revealed that consistent differential fear responding emerged during the last trial of the second acquisition session ($t(66) = 6.36$, $p = 0.0001$), but not between earlier presentations of these stimuli ($t(66) = 0.95$, $p = 0.35$; Figure 10). None of the effects involving the Treatment factor were significant, indicating that nicotine did not differentially affect the rate of acquisition relative to placebo ($F(1, 65) = 0.60$, $p = 0.44$).

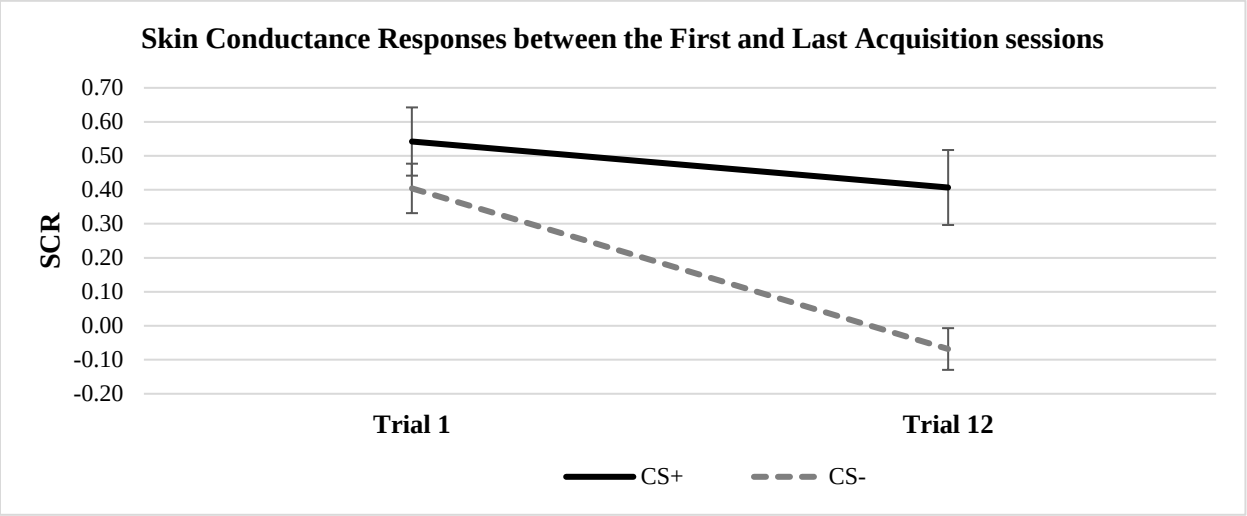


Figure 10. *Differential fear responding emerged consistently during the last trial of the second delay cued acquisition session, but not during the first trial (t(66) =0.95, p = 0.35).*

Contextual test session: Paired sample t-tests were first conducted to examine our *a priori* hypothesis that each treatment group, respectively, would demonstrate greater fear to the virtual context previously paired with a shock (CS+) relative to the safe context (CS-). We found that nicotine-treated participants demonstrated significantly greater SCRs to the CS+ relative to the CS- averaged across trials (M_{CS+}= 0.46 ± 0.78, M_{CS-} = 0.13 ± 0.32; $t(28) = 2.32$, $p = 0.03$). Placebo-treated participants did not show conditioned fear to the CS+ relative to the CS- (M_{CS+} = 0.28 ± 0.60, M_{CS-} = 0.18 ± 0.32; $t(38) = 1.43$, $p = 0.16$).

To test whether nicotine enhances contextual fear conditioning, SCR data were analyzed using a Trial (2) X Stimulus Type (CS+, CS-) repeated measures ANOVA with Treatment (nicotine, placebo) as the between-subjects factor. The ANOVA produced significant main effects of Stimulus Type ($F(1, 65) = 8.45$, $p = 0.005$) and Trial ($F(1, 65) = 4.52$, $p = 0.04$), as well as a Trial x Treatment ($F(1, 65) = 4.08$, $p = 0.05$; Figure 11). As expected, mean SCR magnitude was greater for CS+ (M = 0.36 ± 0.69) relative to CS- trials (M = 0.16 ± 0.31), indicating that participants exhibited greater conditioned fear to the context previously paired

with the aversive shock relative to the neutral, shock-free context. Raw SCR scores were converted to difference scores for post-hoc analysis of the Trial x Treatment interaction by subtracting SCRs to the CS- from those to the CS+. Independent sample t-tests between SCR difference scores revealed that compared to placebo, SCR magnitude was significantly enhanced by nicotine during the first trial of the context test ($M_{nicotine} = 0.33 \pm 0.79$, $M_{placebo} = 0.04 \pm 0.57$; $t(65) = 1.73$, $p = 0.04$, one-tailed; Figure 11). There were no significant differences in contextual fear between treatments for the second trial ($M_{nicotine} = 0.32 \pm 0.93$, $M_{placebo} = 0.17 \pm 0.79$; $t(65) = 0.76$, $p = 0.45$; Figure 11). To ensure that nausea did not disrupt fear conditioning, a multivariate ANOVA was conducted with nausea as a covariate, SCR difference score as the dependent variable and treatment as the between subjects factor. When controlling for nausea, nicotine significantly enhanced conditioned fear on trial 1 ($F(1, 62) = 3.76$, $p = .05$), but had no effect on trial 2 ($F(1, 62) = 0.086$, $p = 0.77$), or the average across trials ($F(1, 62) = 1.66$, $p = 0.20$).

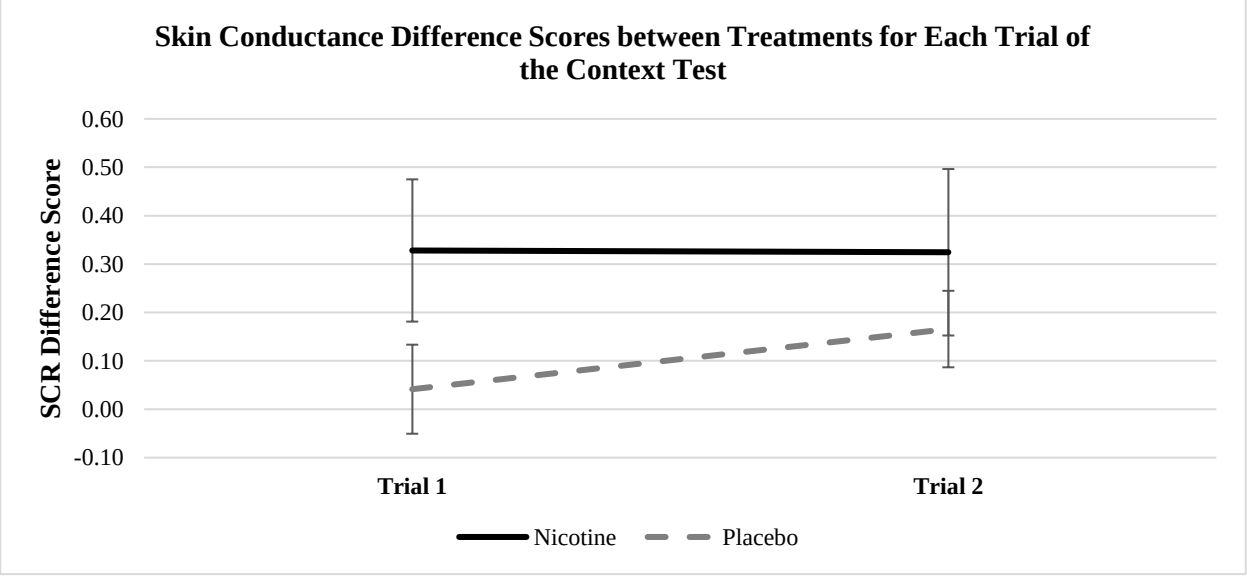


Figure 11. *Relative to placebo, nicotine significantly enhanced fear to the context during the first trial of the context test session (t(65) = 1.73, p = 0.04, one-tailed). There were no significant differences between treatments in contextual fear for the second trial (t(65) = 0.76, p = 0.45).*

Delay cued test session: Paired sample t-tests were first conducted to examine our *a priori* hypothesis that each treatment group, respectively, would demonstrate greater fear to the cue previously paired with a shock (CS+) relative to the safe cue (CS-). Nicotine-treated participants demonstrated significantly greater SCRs to the CS+ cue relative to the CS- cue averaged across trials (M_{CS+} = 0.46 ± 0.67, M_{CS-} = 0.20 ± 0.31; $t(28) = 2.20$, $p = 0.04$; Figure 12). Placebo-treated participants, however, did not exhibit conditioned fear to the CS+ cue (M_{CS+} = 0.51 ± 0.53, M_{CS-} = 0.35 ± 0.80; $t(37) = 1.15$, $p = 0.26$; Figure 12). Examining the effect of nicotine on cued fear conditioning relative to placebo, an ANOVA with repeated measures revealed a significant main effect of Stimulus Type ($F(1, 65) = 4.70$, $p = 0.03$; Figure 12) where responses to the CS+ (M = 0.49 ± 0.60) were significantly greater than to the CS- (M = 0.29 ± 0.63). However, none of the effects involving the Treatment factor were significant, indicating that there were no differences between nicotine and placebo on conditioned fear to the cue ($F(1, 65) = 0.60$, $p = 0.44$; Figure 12).

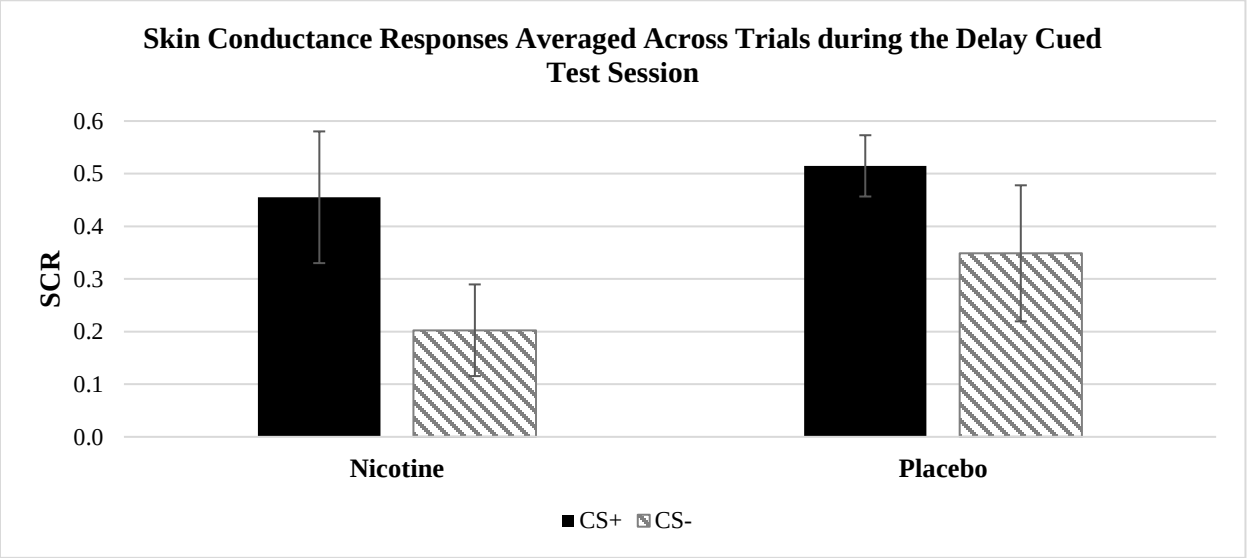


Figure 12. *No significant differences in delay cued fear response between nicotine or placebo treatments ($F(1, 65) = 0.60$, $p = 0.44$).*

Correlations between Questionnaire Metrics and Fear Conditioning: Correlations were conducted to examine potential relationships between delay cued or contextual fear and any of the questionnaire metrics. We hypothesized that individuals with greater levels of nicotine dependence would be more likely to demonstrate nicotine-enhanced contextual FC. Looking across all participants, no significant correlations were found between the Fagerstrom Test for Nicotine Dependence (FTND) and context difference scores during the first (r = -0.62, p = 0.62) nor second trials (r = -0.34, p = 0.79). When specifically examining individuals who received nicotine, no significant relationships were found between FTND and context FC during the first (r = -0.21, p = 0.29) nor second trials (r = -0.15, p = 0.45).

We also hypothesized that individuals who scored highly on measures of stress and anxiety would be more likely to demonstrate learned fear. Examining all participants or those who were administered nicotine, no relationships were found between any subscale (anxiety, depression, or stress) of the Depression Anxiety Stress Scales and contextual FC during any trial ($p > 0.05$). However, in examining all participants, there was a significant positive correlation between the Trait Anxiety subscale of the State Trait Anxiety Inventory and delay cued fear when averaged across trials (r = -0.25, p = 0.04). The Trait Anxiety subscale defines a general tendency to perceive situations as threatening, which differs from State Anxiety which assesses temporary anxiety experienced in specific situations or in the present moment. Furthermore, when specifically examining individuals who received nicotine, delay cued FC significantly correlated with both Trait Anxiety when averaged across trials (r = -0.51, p = 0.01) and State Anxiety during the first CS presentations (r = -0.38, p = 0.05).

4.3.3. Trace Cued and Contextual Fear Conditioning: Of the eighty-three undergraduates recruited for this study, data from fifteen participants were excluded due to participant failure to

follow directions (n = 3), poor physiology recordings resulting from weak electrode adhesion or other recording artifacts (n = 5), or a lack of SCR response to the US as defined by failure to exhibit positive response magnitudes (greater than 0) to the US during the SIR (n = 7). There was no statistical difference between nicotine and placebo groups in attrition (n = 8 (9.6%) in the nicotine group and n = 7 (8.4%) in the placebo group; $p > 0.05$). Sixty-eight participants were included in the final analysis ($n_{nicotine}$ = 39, 9 female, average age = 19.2 ± 1.27; $n_{placebo}$ = 29, 8 female, average age = 19.2 ± 1.19). There were no significant differences between treatment groups in age ($t(65) = -0.30$, $p = 0.77$), hours since last nicotine use ($M_{nicotine}$ = 17.8 ± 18.6, $M_{placebo}$ = 22.7 ± 33.9; $t(65) = -0.76$, $p = 0.45$), or weekly nicotine consumption ($M_{nicotine}$ = 9.15 ± 11.8 $M_{placebo}$ = 15.8 ± 5.87; $t(65) = -0.62$, $p = 0.53$). There were also no differences between treatments on any of the questionnaire measures (Table 14): Fagerstrom ($M_{nicotine}$ = 2.41 ± 2.52, $M_{placebo}$ = 2.07 ± 2.29; $t(65) = 0.56$, $p = 0.58$), DASS Stress ($M_{nicotine}$ = 7.64 ± 7.73, $M_{placebo}$ = 9.29 ± 6.58; $t(65) = -0.91$, $p = 0.36$), DASS Anxiety ($M_{nicotine}$ = 7.64 ± 7.59, $M_{placebo}$ = 8.50 ± 5.90; $t(65) = -0.55$, $p = 0.62$), DASS Depression ($M_{nicotine}$ = 7.03 ± 9.88, $M_{placebo}$ = 5.36 ± 4.83; $t(65) = 0.82$, $p = 0.41$), STAI ($M_{nicotine}$ = 27.0 ± 11.3, $M_{placebo}$ = 26.7 ± 9.34; $t(65) = 0.12$, $p = 0.91$; Table 14). There were also no differences between treatments on the post-test nausea measure ($M_{nicotine}$ = 3.36 ± 3.16, $M_{placebo}$ = 2.93 ± 3.39; $t(63) = 0.53$, $p = 0.60$; Table 14).

Questionnaire Measure	Nicotine (M ± SD)	Placebo (M ± SD)	*t*	*df*	*p*
Fagerstrom	2.41 ± 2.52	2.07 ± 2.29	0.56	65	0.58
DASS Stress	7.64 ± 7.73	9.29 ± 6.58	-0.91	65	0.36
DASS Anxiety	7.64 ± 7.59	8.50 ± 5.90	-0.55	65	0.62
DASS Depression	7.03 ± 9.88	5.36 ± 4.83	0.82	65	0.41
STAI	27.0 ± 11.3	26.7 ± 9.34	0.12	65	0.91
Nausea	3.36 ± 3.16	2.93 ± 3.39	0.53	65	0.60

Table 14. *No significant differences between treatment groups on pre-test questionnaire measures or post-test nausea measure ($p > 0.01$).*

Acquisition: To examine the effect of treatment on the acquisition of conditioned fear across both acquisition sessions, SCR data were analyzed using a 2-factor Trial (1-12) X Stimulus Type (CS+, CS-) repeated measures ANOVA with Treatment (nicotine, placebo) as the between-subjects factor. Analysis yielded significant Stimulus Type ($F(1, 66) = 28.1$, $p < 0.0001$) and Trial main effects ($F(6.03, 397.9) = 3.25$, $p = 0.004$), and a significant Trial x Stimulus Type interaction ($F(6.93, 457.4) = 5.01$, $p < 0.0001$; Figure 13). Participants exhibited conditioned responding as defined by a larger mean SCR magnitude to the CS+ (M = 0.29 ± 0.38) compared to the CS- (M = -0.12 ± 0.23). Adjusting alpha to 0.01 to account for multiple tests, a series of paired t-tests were conducted to examine the Trial x Stimulus Type interaction. Comparing SCR magnitude for CS+ versus CS- trial pairings revealed differential conditioned fear responding ($p < 0.01$) on all trials except for trial 1 ($t(67) = 2.03$, $p = 0.05$), trial 3 ($t(67) = 1.72$, $p = 0.09$), trial 4 ($t(67) = 0.67$, $p = 0.50$), trial 9 ($t(67) = 1.80$, $p = 0.08$), and trial 10 ($t(67) = 0.07$, $p = 0.94$). None of the effects involving the Treatment factor were significant, indicating that nicotine did not differentially affect the acquisition of conditioned responding relative to placebo ($F(1, 66) = 0.01$, $p = 0.93$).

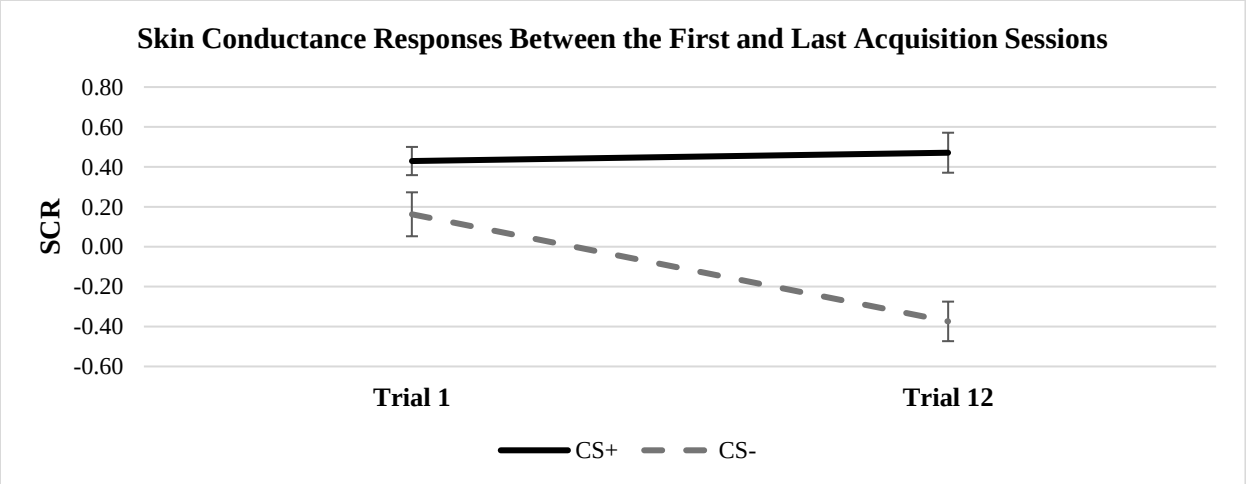


Figure 13. *Differential conditioned fear responding observed during the last trial of the second acquisition session ($\underline{t}(67) = 5.28$, $\underline{p} < 0.0001$), but not during the first trial of the first acquisition session ($\underline{t}(67) = 2.03$, $\underline{p} = 0.05$) .*

Contextual test session: Examining *a priori* hypotheses that each treatment group would respectively demonstrate discriminative fear to the previously shock-paired context, paired sample t-tests revealed significantly greater SCRs to the CS+ relative to the CS- for both the nicotine group (M_{CS+}= 0.45 ± 0.62, M_{CS-} = 0.05 ± 0.31; $t(38) = 5.14$, $p < 0.0001$) and placebo group (M_{CS+} = 0.26 ± 0.31, M_{CS-} = 0.03 ± 0.24; $t(38) = 3.77$, $p = 0.001$) averaged across trials.

To test the effect of nicotine on contextual fear conditioning, SCR data were analyzed using a Trial (2) X Stimulus Type (CS+, CS-) repeated measures ANOVA with Treatment (nicotine, placebo) as the between-subjects factor. The ANOVA produced significant main effects of Stimulus ($F(1, 66) = 15.9$, $p < 0.0001$) and Trial ($F(1, 66) = 4.48$, $p = 0.04$) where the mean SCR magnitude was greater for CS+ (M = 0.37 ± 0.51) than to the CS- (M = 0.04 ± 0.28), and responses decreased across trials. None of the effects involving the Treatment factor were significant, indicating that nicotine did not enhance contextual fear relative to placebo using the trace paradigm ($F(1, 66) = 1.45$, $p = 0.23$).

No effect of treatment was found when using nausea as a covariate ($F(1, 63) = 1.01$, $p = 0.32$). Moreover, there was no effect of treatment on contextual fear when specifically selecting individuals who were aware of the CS-US contingency (awareness score ≥ 4 out of 5; $t(39) = 0.68$, $p = 0.54$). However, in exploratory analysis examining individuals with some level of nicotine dependence (FTND ≥ 1; $n_{nicotine} = 27$, $n_{placebo} = 19$), SCR difference scores were significantly enhanced by nicotine across trials ($M_{nicotine} = 0.51 \pm 0.53$, $M_{placebo} = 0.23 \pm 0.35$; $t(44) = 1.97$, $p = 0.05$; Figure 14).

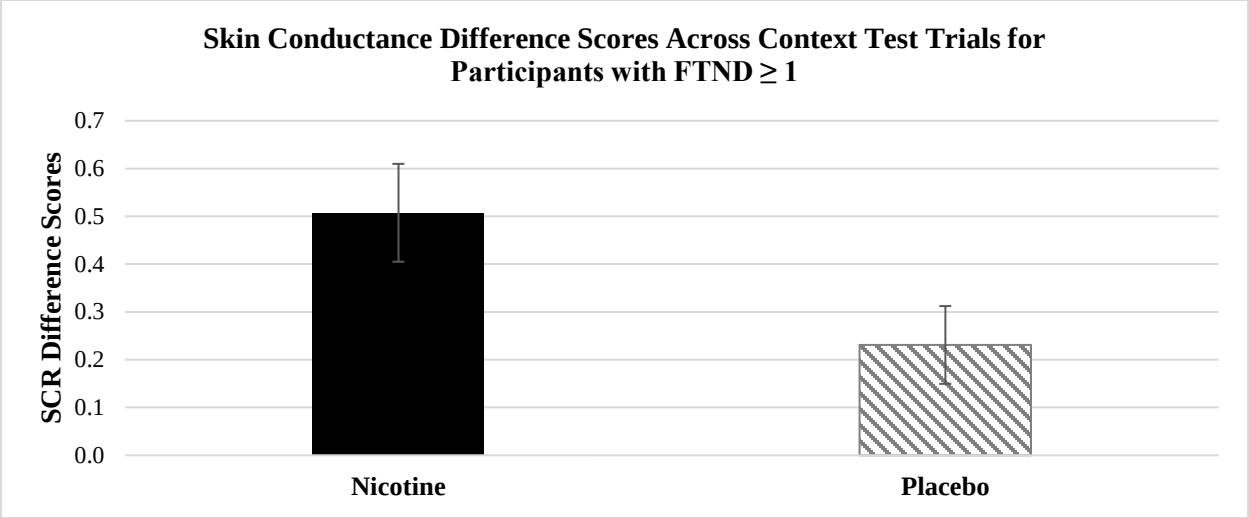


Figure 14. *Nicotine enhances contextual fear for participants with mild nicotine dependence (t(44) = 1.97, p = 0.05).*

Trace cued test session - Paired sample t-tests were first conducted to examine our *a priori* hypothesis that each treatment group, respectively, would demonstrate greater fear to the cue previously paired with a shock (CS+) relative to the safe cue (CS-). Paired sample t-tests revealed significantly greater SCRs to the CS+ cue relative to the CS- cue averaged across trials for both nicotine-treated (M_{CS+} = 0.56 ± 0.86, M_{CS-} = 0.19 ± 0.33; $t(38) = 2.84$, $p = 0.007$) and placebo-treated participants (M_{CS+} = 0.58 ± 0.62, M_{CS-} = 0.18 ± 0.40; $t(28) = 3.88$, $p = 0.001$; Figure 15). Examining the effect of nicotine on trace cued fear conditioning relative to placebo, an ANOVA with repeated measures revealed significant main effects of Stimulus Type ($F(1, 66) = 19.4$, $p < 0.0001$) and Trial ($F(1, 66) = 28.5$, $p < 0.0001$), and a Trial x Stimulus x Treatment interaction ($F(1, 66) = 6.94$, $p = 0.01$), where the magnitude of the conditioned response differed between treatments across trials. Mean SCR magnitude was significantly greater to the CS+ (M = 0.57 ± 0.76) than the CS- (M = 0.19 ± 0.36; Figure 15).

However, there was no between subjects effect of treatment on trace cued fear ($F(1, 66) = 0.008$, $p = 0.93$; Figure 15), suggesting that nicotine did not modify fear responses relative to

placebo. Moreover, no significant finding between treatments was found among participants aware of the CS-US contingency (contingency score ≥ 4; $t(39) = -0.31$, $p = 0.76$), nor among individuals with some level of nicotine dependence (FTND ≥ 1; $t(44) = 0.69$, $p = 0.49$). Given that contingency awareness has been shown to affect the expression of trace conditioning, exploratory bivariate correlations were conducted to determine if there was any relationship between contingency awareness and SCR difference scores during the trace cued test session. However, we found no significant associations between contingency and difference scores on the first trial ($r = 0.08$, $p = 0.54$) nor second trial of the trace cued test ($r = 0.18$, $p = 0.14$), or when averaged across trials ($r = 0.13$, $p = 0.28$).

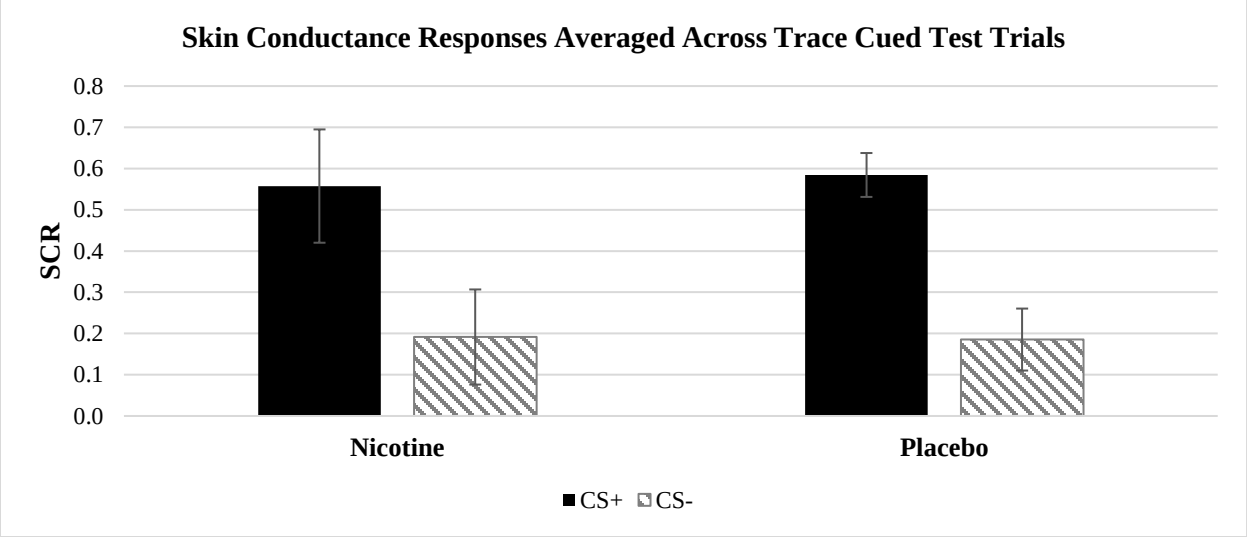


Figure 15. *Trace cued fear learning found for both the nicotine ($t(38) = 2.84$, $p = 0.007$) and placebo ($t(28) = 3.88$, $p = 0.001$) groups. However, no significant differences between treatments in trace cued fear ($F(1, 66) = 0.008$, $p = 0.93$).*

Correlations between Questionnaire Metrics and Fear Conditioning: Correlations were conducted to examine potential relationships between trace cued or contextual fear and any of the questionnaire metrics. Given that contingency awareness has been shown to affect the expression of trace conditioning, bivariate correlations were conducted to determine if a relationship existed between contingency awareness and SCR difference scores during the trace

cued test session. However, we found no significant associations between contingency and difference scores on the first trial ($r = 0.08$, $p = 0.54$) nor second trial of the trace cued test ($r = 0.18$, $p = 0.14$), or when averaged across trials ($r = 0.13$, $p = 0.28$).

We hypothesized that individuals with greater levels of nicotine dependence would be more likely to demonstrate nicotine-enhanced contextual and trace FC. Looking across all participants, no significant correlations were found between the Fagerstrom Test for Nicotine Dependence (FTND) and context difference scores during the first ($r = -0.02$, $p = 0.93$) nor second trials ($r = 0.05$, $p = 0.77$). There were also no relationships between FTND and trace cued difference scores during the first ($r = -0.04$, $p = 0.79$) nor second trials ($r = 0.06$, $p = 0.70$). When specifically examining individuals who received nicotine, no significant relationships were found between FTND and context FC ($p > 0.05$) nor trace cued FC ($p > 0.05$).

We also hypothesized that individuals who scored highly on measures of stress and anxiety would be more likely to demonstrate learned fear. Examining all participants or those who were administered nicotine, no relationships were found between any subscale (anxiety, depression, or stress) of the Depression Anxiety Stress Scales and contextual or trace FC during any trial ($p > 0.05$). However, there was a significant positive correlation between the State Anxiety subscale of the State Trait Anxiety Inventory and trace cued fear when averaged across trials, specifically for participants who received nicotine ($r = 0.34$, $p = 0.04$). The State Anxiety subscale assesses temporary anxiety experienced in specific situations or in the present moment, which differs with Trait Anxiety which defines a general tendency to perceive situations as threatening.

4.4. Discussion

The aim of the present study was to extend nonhuman findings of nicotine-enhanced hippocampal-based fear learning to humans. We found that nicotine enhanced contextual fear but had no effect on delay cued conditioning. Our findings are consistent with abundant nonhuman reports that acute nicotine enhances contextual but not delay cued fear learning (Gould & Wehner, 1999; Gould & Higgins, 2003; Wehner et al., 2004; Davis et al., 2006b, 2007, Gould & Lommock, 2003, Gulick & Gould, 2008, Portugal et al., 2012).

Since both human (see review: Davis & Whalen, 2001; Cheng et al., 2003; Alvarez et al., 2008; Knight et al., 2004) and non-human studies (Phillips & LeDoux, 1992; Rudy et al., 2004; Paré et al., 2004) indicate that the hippocampus mediates contextual conditioning, but is not involved in delay cued conditioning, nicotine likely has an affinity for hippocampal-dependent versions of learning. Further support for this theory comes from others who have demonstrated that nicotine also enhances hippocampal-dependent spatial learning, where acute nicotine administration improves choice accuracy on the radial arm maze (Levin & Rose, 1991; Levin, 1997) and improves performance on the Morris water maze (Brown et al., 2000, 2001; Decker et al., 2002; Socci et al., 1995). Given that trace fear conditioning is also hippocampus dependent, it should also be enhanced be nicotine (Gould et al., 2004; Crestani et al., 2002; McEchron et al., 1998; Quinn et al., 2002). Several nonhuman studies indicate that nicotine does enhance trace conditioning (Gould et al., 2004; Raybuck & Gould, 2009; Davis et al., 2006b). However, while both nicotine- and placebo-treated participants in the present study demonstrated successful trace conditioning, we found no enhancement by nicotine on trace cued fear in humans.

Some have suggested that contingency awareness is a prerequisite for successful conditioning in humans. Contingency awareness implies that participants can communicate the

association between CS and US. Several studies have failed to observe differential SCR conditioning in participants unaware of the CS-US contingency using a delay cued paradigm (Dawson et al., 1986, 2007; Hamm & Vaitl, 1996; Purkis & Lipp, 2001; Tabbert et al., 2006; Weike et al., 2006; Kucken et al., 2009). Others, however, have determined that delay cued conditioning occurs independent of contingency awareness (Clark & Squire, 1998; Manns et al., 2001; Schultz & Helmstetter, 2010; Knight et al., 2003, 2006). Despite discrepant findings regarding the relationship between awareness and Pavlovian conditioning for delay cued FC, it is generally agreed that contingency awareness is necessary for trace conditioning paradigms since the trace period increases the complexity of the task (Clark & Squire, 1998; Manns et al., 2000a, 2000b; Clark et al., 2001; Knight et al., 2006; Weike et al., 2007; Klucken et al., 2009).

The role of awareness in delay and trace conditioning was assessed by Knight et al. (2006), who recorded SCRs while manipulating the intensity of an auditory CS on a trial-by-trial basis to create perceived and unperceived trials. For the delay group, the CS+ co-terminated with a loud 500ms white noise. For the trace group, the CS+ was separated from the white noise US by a 4.5-second trace interval. Perception of the CS was monitored by asking participants to click the mouse immediately upon detecting the tone. Participants then used the mouse rate their expectancy of the US on a continuous scale from 0 to 100. Differential expectancies of the US were found only for perceived delay and trace conditioning trials. Examining learning-associated changes in SCR, differential conditioning was observed for both perceived and unperceived delay cue presentations; however, differential SCRs were only observed for perceived trace conditioning trials. They therefore concluded that while delay conditioning develops without explicit contingency awareness, trace conditioned responding requires awareness (Knight et al., 2006). Moreover, their findings may reflect a dissociation between declarative and non-

declarative cognitive systems.

Despite evidence that contingency awareness is necessary for CR expression of trace conditioning, we found no significant association between awareness and trace cued conditioning in the present study. Both the nicotine and placebo groups acquired differential trace conditioning independent of contingency awareness. Moreover, nicotine did not enhance trace conditioning even among those with a high degree of CS-US awareness. It is possible that our post-test contingency awareness questionnaire may have been an inadequate measure of CS discrimination, as it has been suggested that post-conditioning questionnaires may be susceptible to forgetting, interference, and reconstruction of memory prior to awareness evaluation (Lovibond & Shanks, 2002; Schultz & Helmstetter, 2010). Therefore, a trial-by-trial measure like that used by Knight et al. (2003, 2006) may be more sensitive. Nonetheless, the degree of contingency awareness cannot explain our lack of finding nicotine-enhanced trace conditioning, and other possibilities must be considered.

Interestingly, while acute nicotine enhances hippocampal-mediated fear conditioning, chronic nicotine treatment does not alter fear conditioning, even when the dose administered produces plasma nicotine levels equivalent to those of acute treatments that facilitate enhanced contextual conditioning (Davis et al., 2005; Portugal et al., 2012). Moreover, these plasma nicotine levels are comparable to those exhibited by smokers (Benowitz et al., 1989; Henningfield et al., 1985, 1993). It is possible that the neural adaptations that occur with chronic nicotine use result in tolerance for nicotine's effects on contextual conditioning. While no studies to our knowledge have investigated the effect of chronic nicotine on trace conditioning, it could be assumed that chronic use may also disrupt this variant of hippocampal-dependent learning. Given that the present study was conducted in pre-existing nicotine users, it is possible that our

investigation of the effects of acute nicotine on trace cued conditioning was confounded by chronic nicotine use. However, we saw significant nicotine enhancement of contextual fear in the same sample of participants, particularly in those with greater levels of nicotine dependence who logically use more frequently, discrediting this hypothesis.

Therefore, the most likely explanation for our lack of finding nicotine-enhanced trace conditioning is paradigmatic differences between our study and those conducted preclinically. In studies conducted by Gould and colleagues, the enhancing effects of nicotine on hippocampus dependent fear learning were observed when nicotine was administered prior to both training and testing (Gould, 2003; Gould & Higgins, 2003; Gould & Lommock, 2003; Gould et al., 2004; Davis et al., 2006b; Raybuck & Gould, 2009). Importantly, the effect of nicotine-enhanced contextual fear disappeared when nicotine was given only on training day, or only on testing day. Thus, the effects of nicotine on neural processes present during both conditioning and testing seem vital for enhanced conditioning. Since studies have not investigated whether nicotine must be administered at training and testing in order to facilitate trace conditioning, one cannot rule out the possibility.

In the present study, nicotine was only administered prior to the acquisition of conditioning; therefore, it is possible that a lack of repeated nicotine administration before the test session disrupted participants' ability to access the trace fear memory. Our test sessions were estimated to have occurred 30-40 minutes after nicotine administration. It has been previously reported that the effects of the 2mg nicotine lozenge peak 15-20 minutes after administration (McEwen et al., 2008; Choi et al., 2003). However, plasma nicotine levels remain stably elevated for at least 40 minutes before decreasing to baseline (McEwen et al., 2008; Choi et al., 2003). Therefore, nicotine was likely still on-board and at peak latency during the test session to

influence the neural processes required for trace cued fear enhancement. Moreover, a single nicotine administration prior to training was enough to enhance contextual fear in both the delay and trace paradigms. Therefore, further investigation is needed to examine the importance of nicotine administration prior to both training and testing.

Additionally, different routes of administration between our study and those conducted in nonhumans should also be noted. To our knowledge, no studies have investigated whether different routes of nicotine administration modify conditioned fear responses in nonhumans. However, since routes of administration have been deemed to variably effect nicotine-enhanced reinstatement in humans (see section *2.4.*), it is likely that they may also influence aversive conditioning, and thus require further research. To summarize aforementioned studies, Perkins and colleagues have shown across a multitude of studies that the effect of acute nicotine on human operant responses for money, music, or the termination of aversive noise varies depending on the route of nicotine administration. For example, reinforced responding due to nicotine via spray or patch was greater for video reward, but not for music reward (Perkins et al., 2018), while acute nicotine intake from smoking enhanced the reinforcing value of music rewards, but not any other reward type (Perkins & Karelitz, 2013).

Contrary to our *a priori* hypotheses, placebo-treated participants in the present study did not exhibit conditioned fear to the cue or context during the delay fear paradigm. Studies have shown that mice 24-hours withdrawn from chronic nicotine treatment demonstrate impaired contextual fear conditioning compared to those treated with saline, and this deficit can be reversed with an acute nicotine challenge (Davis et al., 2005; Davis & Gould, 2007, 2009; Portugal & Gould, 2007; Portugal et al., 2008). While withdrawal is the simplest explanation for failing to find cued and contextual fear among placebo-treated participants, it is unlikely that our

nondependent participants (average FTND = 2.97 ± 2.70) were experiencing withdrawal symptoms after willingly abstaining from nicotine use for longer than the required 6-hour window of abstinence (average time since last use = 11.7 ± 8.28 hours). Unfortunately, we did not have the foresight to collect a measure of withdrawal; therefore, we cannot scientifically characterize withdrawal symptoms in the present sample. However, while our participants were likely not experiencing classic withdrawal symptoms of irritability, anxiety, autonomic symptoms, etc. (DSM–V; American Psychiatric Association, 2013), it is possible that they were experiencing some cognitive deficit that prevented them from accessing fear memories in the absence of nicotine, which has been shown to enhance cognitive processes and attention in humans (Young et al., 2004). If this were the case, nicotine may not necessarily enhance fear learning, but instead attenuate abstinence-induced deficits in the brain reward system. This theory may be answered by further exploration of the relationship between withdrawal and conditioning in a more dependent sample.

The direct effects of nicotine on hippocampal-mediated learning has implications on addictive processes since nicotine may facilitate the development of maladaptive drug-context associations, leading to enhanced cravings and subsequent drug-seeking behavior. Interestingly, research has attempted to localize the effects of nicotine within the hippocampus, where discrete injections into the ventral and dorsal hippocampus, respectively, yield distinct functional outcomes. Specifically, localized nicotine infusions into the dorsal hippocampus resulted in an enhancement of contextual fear learning, whereas nicotine infused into the ventral hippocampus impaired conditioning and extinction (Kenney et al., 2012; Kutlu et al., 2018). As discussed, the dorsal hippocampus has been associated with contextual and spatial learning. The ventral hippocampus, however, has been implicated in processes of anxiety, stress, and emotional

alteration (Fanselow & Dong, 2010; Gould & Leach, 2014). While acute nicotine administration may enhance dorsal hippocampus-mediated processes resulting in a facilitation of learning, manipulations that favor processes mediated by the ventral hippocampus may be disadvantageous, ultimately contributing to anxiety disorder pathology.

In examining correlations between AD and fear learning, we found significant positive correlations between delay cued fear and both the Trait and State anxiety subscales, respectively, of the State Trait Anxiety Inventory (STAI) for nicotine-treated participants. Trace cued fear was also significantly associated with State anxiety for nicotine-treated participants. In other words, individuals with greater tendencies to present anxiety symptoms generally, and in specific situations, are more likely to demonstrate enhanced fear learning to conditioned stimuli. As mentioned previously, aberrant Pavlovian conditioning mechanisms may be responsible for the development of AD (Morrow et al., 2011; Bush et al., 2007), and marked comorbidity between AD and nicotine dependence has been reported (Lasser et al., 2000; Ziedonis et al., 2008; Grant et al., 2006). Moreover, it has been suggested that while nicotine use may attenuate symptoms of anxiety short-term (Robinson et al., 2009), it may also predispose individuals to AD by enhancing baseline symptom severity over time (Kutlu & Gould, 2015; Kutlu et al., 2015). Therefore, the present findings substantiate the use of the fear conditioning model to investigate vulnerability to AD and provide a foundation for future studies aimed at characterizing specific brain systems that mediate the interaction between nicotine dependence and anxiety.

The present study is the first to investigate the effects of nicotine on fear conditioning in humans. Overall, we found that nicotine enhances contextual fear, but has no effect on cued fear conditioning, substantiating nonhuman findings of specific enhancement of hippocampal-dependent fear. However, we observed no effect of nicotine on hippocampal-mediated trace cued

fear, necessitating further investigation. Given the comorbidity of nicotine dependence and AD, this translational research provides an important foundation for critical understanding of the development, maintenance, and relapse to nicotine use that is important to nicotine users with anxiety disorders.

V. GENERAL DISCUSSION

5.1. Nicotine reward enhancement using the conditioned place preference paradigm

The effects of nicotine on cognition may facilitate the development and maintenance of nicotine dependence through multiple mechanisms. These data demonstrate that nicotine partially enhances human sensitivity to reward using a virtual conditioned place preference paradigm. Within treatments, the nicotine group demonstrated a CPP by spending significantly more time in the room previously paired with a chocolate food reward and the placebo group did not. However, we did not observe significant differences between treatment groups, such that the nicotine group did not spend significantly more time in the rewarded room than did placebo.

Findings from this study suggest that those with a mild level of nicotine dependence are more likely to demonstrate nicotine-enhanced CPP than those with lesser or no indices of dependence. Moreover, these findings indicate a potential relationship between withdrawal and blunted reward sensitivity, perhaps hindering CPP in placebo-treated participants. However, we did not have the foresight to collect a measure of nicotine withdrawal, and because our sample was comprised of light nicotine users who willingly abstained from nicotine use for longer than the 6-hour requirement, the confounding effects of withdrawal are unlikely. Therefore, future studies should more intensively characterize the relationship between withdrawal and reward enhancement, particularly in individuals with higher indices of nicotine dependence.

These findings also suggest that discrepancies in reward enhancement between

preclinical and clinical studies may result from paradigmatic differences between experiments. The present study highlights a role for memory consolidation in nicotine-enhanced conditioning, where multiple day conditioning paradigms may be needed to observe enhanced responding. Moreover, nicotine administration may be needed both pre-conditioning and at testing to successfully acquire enhanced conditioning and subsequently access the memory. However, the timeline of these mechanisms remains to be investigated in humans. Therefore, the exploration of nicotine administration timing and multiple day paradigms is needed.

Finally, the present study underscores the needs to further examine the role of nicotine delivery route and reward type specificity in nicotine-enhance Pavlovian conditioning. It is possible that the 2mg lozenge used in the present study results in a lesser degree of reward enhancement relative to other routes of nicotine delivery, like smoking or intravenous delivery. Moreover, operant conditioning studies in humans suggest that nicotine reinforcement enhancement is more prominent for some sensory rewards than others. Therefore, it would be of interest to utilize the present model to examine whether route of delivery or reward type specificity play a role in classical conditioning as well.

These novel data extend nonhuman findings of reward enhancement and have important relevance to the field of substance abuse as they pertain to how Pavlovian conditioning factors generalize to humans. The ability of nicotine to facilitate consummatory behavior controlled by non-nicotine stimuli may be one factor that contributes low rates of successful smoking cessation. Therefore, evidence that nicotine magnifies the incentive salience of rewarding places increased importance on therapeutic strategies that target both nicotine use and the rewarding value of associated stimuli and contexts.

5.2. Differences in reward processing between nicotine users and non-users

Directly comparing the magnitude of incentive salience attributed to nicotine and non-nicotine rewards between nicotine users and non-users, the present data add evidence to a growing body of literature that nicotine users attribute enhanced incentive salience to nicotine-related rewards relative to non-users. However, little evidence of hyporeactivity to non-nicotine rewards among nicotine users was observed, suggesting that enhanced motivational processing for drug rewards does not attenuate the reward value of non-drug cues. This finding is in line with several other clinical studies that also fail to show evidence of reduced motivation for non-drug rewards among nicotine users and clarifies discrepancies within the literature where the magnitude of incentive salience devoted to drug and non-drug rewards is incompletely understood.

Interestingly, we found that among nicotine users, nicotine cues were less arousing than negative cues with no differences in physiological responses between images. These novel findings indicate that the magnitude of incentive salience attributed to nicotine rewards may be dependent upon affective stressors, where nicotine only biases associative processes toward rewarding cues to the degree that these cues are of equal salience. In this sense, the incentive sensitizing effects of nicotine may not be able to overcome affect-inducing demands of highly salient-negative stimuli. Given that negative affect plays a key role in nicotine craving and relapse, future studies should further investigate the ability of nicotine to bias salience attribution toward drug cues when motivational processes compete with highly salient negative affective stimuli.

In line with previous human and nonhuman work, our data also dissociate pleasurable "liking" from motivated "wanting," by demonstrating that valence ratings for all image types did not correlate with physiological responses. While both systems are controlled by

mesocorticolimbic structures, neurobiological evidence indicates that distinct neural substrates mediate components of "liking" and "wanting." However, since hedonic responses to nicotine predict future use, it is clinically relevant to assess how the "liking" and "wanting" systems work together to process reward and contribute to nicotine dependence. Therefore, future human work should investigate both components of reward processing when characterizing the role of nicotine in motivated behavior.

In addition to characterizing differences between nicotine users and non-users, our findings also revealed that acute nicotine administration did not influence motivational processing in our nondependent sample. Those who have demonstrated increased reward responsiveness following acute nicotine have largely focused on nicotine's ability to attenuate abstinence-induced deficits, suggesting that nicotine dependent individuals may be inherently hyposensitive to reward, predisposing them to seek nicotine to overcome such deficits. If nicotine enhances reward processing as a function of withdrawal deficit reversal, then it is possible that our sample was not dependent enough to experience withdrawal symptoms, obscuring differences between treatments. Therefore, future studies must employ samples with diverse nicotine dependence levels to better elucidate the role of withdrawal in reward processing.

In a further attempt to describe the role of nicotine in the motivational processing of non-drug rewards, the present study failed to replicate findings from a previous study which found that attractiveness ratings of facial stimuli differ between nicotine users and non-users. Moreover, we found no effect of nicotine on social reward processing relative to placebo. We expect that methodological differences account for discrepancies across studies since different pictorial stimuli and rating scales were used. Importantly, however, our findings again suggest

that route of nicotine delivery may influence reward responsivity. The previous studies administered nicotine via nicotinized and denicotinized cigarettes, while we used a 2mg nicotine lozenge. Therefore, additional research is needed to examine the effects of alternate methods of nicotine consumption on facial attractiveness reward enhancement.

Finally, given evidence that impulsive individuals may be more sensitive to the rewarding and reinforcing effects of nicotine, the present work aimed to determine whether nicotine users would exhibit riskier behavior than non-users on a computerized measure of risk-taking. However, we found no effect of nicotine use status on this task, regardless of whether participants were working for virtual rewards, or working for lottery entries into a drawing for a $20 Amazon gift card. We expect that the reinforcers used in the present study were not salient enough to generate robust responses on this task. Given weak responses, it was unsurprising that this behavioral measure of risk-taking did not correlate with any measure of reward processing. Therefore, future studies should investigate how the magnitude of reward saliency influences behavioral measures of risk-taking by administering real monetary rewards at the time of the experiment. In turn, these measures may be used to examine the relationship between impulsivity and reward responsiveness.

The present study is the first to incorporate the International Affective Picture System and International Smoking Image Series to investigate differences in nicotine cue responsiveness between users and non-users using skin conductance. Our study complements existing literature illustrating that nicotine users and non-users exhibit different profiles of motivational processing for rewards. Additionally, our study is one of few to evaluate salience attribution to nicotine and non-nicotine stimuli within the same model, demonstrating that enhanced motivational processing for drug rewards does not attenuate the reward value of non-drug cues in nicotine

users. Therefore, a detrimental preference for nicotine use seems to result from increased incentive salience to drug rewards, and not from decreased incentive salience to non-drug rewards. Therefore, while nicotine use may not blunt non-drug reward sensitivity, it does still alter the balance between the incentive salience of drug rewards relative to non-drug rewards, and this imbalance may drive subsequent motivation to obtain nicotine. Therefore, preventative approaches or therapeutic interventions should aim to reduce relapse by decreasing physiological and hedonic responses to drug-associated stimuli. It may also be of interest to examine whether enhanced incentive salience of non-drug rewards may restore the incentive salience imbalance, and confer protective mechanisms that reduce nicotine craving and seeking.

5.3. The effect of nicotine on conditioned fear

In addition to enhancing the incentive motivational properties of rewarding cues, nicotine may also enhance the motivational value attributed to aversive cues, where it has been suggested that these aberrant Pavlovian conditioning mechanisms may be responsible for the development of anxiety disorders. The present studies were the first to extend numerous nonhuman reports that acute nicotine enhances contextual, but not delay cued fear learning, to humans. Moreover, these findings elucidate the neural mechanisms through which nicotine affects fear learning, suggesting that nicotine has an affinity for hippocampal-dependent versions of fear learning.

However, we were unable to reproduce preclinical findings of nicotine-enhanced trace fear conditioning, which is also hippocampal-dependent, necessitating further investigations. While some have suggested that contingency awareness is a prerequisite for successful trace conditioning in humans, we found no significant relationship between awareness of the CS-US association and trace cued conditioning. Moreover, nicotine did not enhance trace conditioning when specifically examining individuals with a high degree of awareness. Our findings suggest

that a post-test measure of contingency may not adequately assess CS discrimination, therefore future studies may benefit from employing a trial-by-trial contingency awareness measure.

It is possible that our inability to replicate nicotine-enhanced trace fear may extend from paradigmatic differences between our study and those conducted preclinically. Specifically, further investigation is needed to determine the importance of nicotine administration prior to both training and testing. While no studies have investigated nicotine administration timing on trace fear, nonhuman studies only detected a treatment effect when nicotine was administered prior to both training and testing. While we believe nicotine was still at peak latency during conditioning and testing, dividing the experiment into two consecutive days with n icotine administration prior to each phase may prove effective. This suggestion implies that nicotine facilitates processes that encourage both acquisition and subsequent access of the learned response, and that different neural substrates may be activated by nicotine once memories are learned.

The present findings also elucidate a relationship between AD and nicotine-enhanced fear learning, where individuals with greater tendencies to present symptoms of anxiety are more likely to demonstrate enhanced fear learning to conditioned stimuli. Therefore, the present findings substantiate the use of the virtual fear conditioning model to investigate vulnerability to AD. Examining how nicotine modulates conditioned learning may elucidate whether integrating a plan for nicotine reduction or cessation is critical in designing successful treatments for individuals with AD. Conversely, this research might highlight the need for anxiety-reducing strategies in facilitating nicotine cessation. This research also has implications for better understanding the psychological processes underlying drug relapse. Evidence from the present work suggests that nicotine uniquely enhances hippocampal-dependent contextual learning in

humans; therefore, extinguishing drug-context associations that lead to drug-seeking behavior and cravings may be particularly important when designing relapse intervention strategies.

In addition to informing treatments, this study greatly benefits the research community by functioning as a screening tool for future neuroimaging studies that evaluate the specific brain systems mediating nicotine's differential effects on those with and without anxiety. This could potentially lead to a greater understanding of mechanisms underlying nicotine dependence and anxiety disorder comorbidity, as well as identify differential treatment response.

Finally, evidence from nonhuman studies suggests that acute, chronic, and withdrawal from chronic nicotine have differential effects on hippocampus-dependent and hippocampus-independent fear learning (see review: Kutlu & Gould, 2015). Therefore, our virtual fear conditioning paradigm may be useful for future investigations of the neurobiological mechanisms involved in nicotinic modulation of fear learning at different stages of nicotine use. In sum, these studies provide critical and translational bridges allowing for a more in-depth examination of conditioning and relapse mechanisms across species.

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